A SPECIFIC METABOLIC HORMONE OF THE PITUITARY GLAND AND ITS RELATION TO THE MELANOPHORE-DILATING HORMONE

A SPECIFIC METABOLIC HORMONE OF THE PITUITARY GLAND

AND

ITS RELATION TO THE MELANOPHORE-DILATING HORMONE.

-by-

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THESIS

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INTRODUCTION

The study of the direct role of the pituitary gland in metabolism is obscured by the intricate maze of interrelationships with other endocrine glands. Hence many apparently characteristic signs of hypophyseal deficiency are in reality due to secondary degeneration of dependent glands, particularly the thyroid and adrenal cortex. As a result of the rapid advance in our knowledge of the trophic hormones of the anterior lobe of the pituitary, we are now able to identify, with some degree of certainty, some cardinal signs of hypophyseal deficiency. These are in general more obvious within a few days after hypophysectomy, in contra-distinction to the secondary symptoms which may take as long as three weeks to become fully established, owing to the slow regression in the activity of the dependent gland. The primary signs of pituitary deficiency are also often recognizable by their unique characteristics, not simulated by a deficiency of any other hormone.

Taking cognizance of these facts, it is of interest to discuss the more immediate signs of pituitary deficiency, and pari passu, to discuss the more immediate effects occurring after injection of pituitary extracts.

In this manner one should attain a deeper insight into the direct influence of the pituitary gland on general metabolism.

The scope of this review and the subsequent experimental work involve not merely the anatomical entity referred to as the anterior lobe, but also data on the posterior lobe, which does not include the effect of the true posterior lobe hormones oxytocin and vasopressin. Hence reference is made to "posterior lobe extracts" which include an appreciable portion of the pars intermedia.

Since the literature on the role of the anterior pituitary in metabolism has already been very completely reviewed (Black, 1935; Collip, 1935; Houssay, 1936; Van Dyke, 1936; and Russell, 1938,a) only a brief summary is included in the present work, where special points are emphasized to assist the reader's interpretation of experimental data to follow. Copious references on well-established facts are therefore excluded, since they are readily available in the numerous reviews.

HISTORICAL REVIEW.

Primary Signs of Hypophyseal Deficiency.

Within three days after hypophysectomy animals invariably show a tendency to hypoglycaemia, particularly if during the period of post-operative recovery the food intake is low. In dogs, rabbits, monkeys, guinea pigs, birds and toads this is frequently fatal unless glucose is given. The dramatic effect of glucose in restoring the moribund animal to an apparently normal state suggests that the low blood sugar per se is the primary abnormality. Hypophysectomized rats are rather exceptional, since they withstand fasting better than other animals, and rarely experience hypoglycaemic attacks. Nevertheless, the blood sugar and carbohydrate stores in liver and muscle are at an abnormally low level after 48 hours' fast (Phillips and Robb, 1934; and Russell, 1936). The aetiology of the hypoglycaemia is by no means obvious, but is probably due to abnormal dissipation of the carbohydrate stores. Bennett (1938) by a careful investigation of adrenalectomized and hypophysectomized rats, out-ruled the possibility that this effect could be attributed to secondary adrenal cortical atrophy. A few observations in the literature on other animals

confirm the findings on glycogen stores of fasted rats (Houssay, 1936, on dogs; Corkill, Marks and White, 1933, on rabbits, Houssay, di Benedetto and Mazzocco, 1933, on toads). There are obviously three possible reasons why the hypophysectomized animal should be unable to conserve its glycogen stores: first, a deficiency in gluconeogenesis from either protein or fat; second, a lack of some carbohydrate inhibitory mechanism; and finally, a much neglected possibility of a relative inability to oxidize fat. These three alternatives will recur frequently in the interpretation of various pituitary phenomena. The arguments in support of each will be advanced later. Associated with the loss of carbohydrate in fasted hypophysectomized animals, Fisher and Pencharz (1936) and Fisher, Russell and Cori (1936) found that they had a relatively higher respiratory quotient than fasted controls: .75 and .72 respectively. Similar observations on other animals are scanty owing to the tendency to fatal hypoglycaemia. Artundo (1931) and Gaebler (1929) have reported normal or slightly elevated values in dogs. However, the possibility of incomplete hypophysectomy in this species must be borne in mind (Cushing, 1910). In fact Gaebler (1929) found an

appreciable amount of pars intermedia tissue at the base of the brain in the single case which he reported. Charles (1931) reported studies on the gaseous metabolism of the amphibian Xenopus laevis. She observed a fall in oxygen consumption following hypophysectomy, which was more pronounced if the whole pituitary was removed than after removal of only the pars glandularis. The respiratory quotient after hypophysectomy was elevated, being sometimes as high as 1.09.

Another remarkable characteristic of hypophysectomized animals is the rapid appearance of hypersensitivity to insulin. It was first described by Houssay in 1924, and has since been confirmed in almost every laboratory animal (Russell, 1938,a) with the exception of birds (Sciesinski, quoted by Houssay, 1936, Dunham Lectures). In general such animals respond to one-tenth the normal dose of insulin. Thyroidectomy produces a similar reaction, though not so marked (Ducheneau,1924; Burn and Marks, 1925; Britton and Myers, 1928). However, its onset is relatively slow after removal of this gland, and subsequent removal of the pituitary will increase the sensitivity still more (Cope and Marks, 1934).

The most likely explanation in the case of hypophysectomized animals appears to be the low carbohydrate stores present after a short fast, which would tend to accentuate the hypoglycaemic action of insulin. An alternative explanation is based on the observation that hypophysectomized animals show a diminished hyperglycaemia to adrenalin, and so lose the full benefit of its compensatory control of hypoglycaemia. But this view is less probable as there is now considerable evidence that hypophysectomized animals are as sensitive to intravenous adrenalin as are normals.

The hyperglycaemic response to adrenalin in the absence of the pituitary has been the subject of numerous publications. Corkill, Marks and White (1933) and Cope and Marks (1934) have reported diminished hyperglycaemia in rabbits despite ample stores of liver glycogen (see also Houssay and di Benedetto, 1932; and Kusonoki and Nakamura, 1934). Chaikoff, et al (1935) explained the phenomenon in hypophysectomized dogs by a diminution of the breakdown of muscle glycogen to lactic acid, and therefore a relative inability to replace liver glycogen during the phase of hyperglycaemia. Bachman and Toby (1936) later confirmed the relative inability of hypophysectomized rabbits under the influence of adrenalin to mobilize

muscle glycogen. Collip, Thomson and Toby (1936) pointed out that in hypophysectomized rats this phenomenon was correlated with the fall in B.M.R. due to thyroid atrophy, while pretreatment with thyroxin elicited a normal muscle glycogen response to adrenalin. To exclude the factor of delayed absorption present in animals hypophysectomized some time previously (Bennett, 1936), other workers used intravenous adrenalin. Braier (1931a,)working with dogs, and Russell and Cori (1937 with rats, found no difference in the response of hypophysectomized animals. The latter workers used a slow intravenous injection and studied blood sugar, muscle glycogen and glucose tolerance curves. This series of experiments gives a very lucid example of the difficulties encountered in separating the primary and secondary signs of hypophyseal deficiency.

About 1929 Houssay and coworkers described the alleviation of experimental pancreatic diabetes by hypophysectomy. The discovery was apparently ignored and only in later years have other laboratories hastened to confirm the dramatic observations of the South American physiologist. It has already been repeated on a wide variety of species (dogs, cats, rats, monkeys,

toads and dogfish (Russell, 1938,a)). Following the second operation the blood sugar diminishes appreciably and glycosuria may be abolished. Ketonuria is also diminished or absent. The animals, although clinically by no means normal, may remain alive for more than a year without insulin treatment. On feeding glucose, the animals show a rise in R.Q. suggesting a return of the ability to oxidize glucose (Biasotti, 1934). They resemble the hypophysectomized animal in the tendency to hypoglycaemic crises. The onset of this biochemical improvement after hypophysectomy is so rapid that it outrules the possibility that secondary atrophy of other endocrines might play a role. However, it must be borne in mind that Long and Lukens (1936) have shown that removal of both adrenals in cats will cause a similar amelioration of pancreatic diabetes. A typical diabetes will reappear if adrenal cortical tissue is transplanted into such an animal (Long, 1937). As will be seen later, an extract of pituitary gland has been prepared which will rapidly produce a temporary recrudescence of diabetes in depancreatized-hypophysectomized animals. It therefore appears legitimate to claim that the pituitary normally secretes a hormone having a diabetic-like action on metabolism.

The Houssay phenomenon has provoked some interesting discussion. The views adopted by various workers are naturally coloured by their conception of pancreatic diabetes. Soskin, et al (1935) have attributed the effect to an inhibition of excessive gluconeogenesis which has been postulated in the over-production theory of diabetes. They held that the pituitary plays a role in the conversion of fat to carbo-hydrate. Since only gluconeogenesis from protein remains, the glycosuria is diminished. They pointed out that a Houssay dog shows glycosuria on a high protein diet, but not on a high fat diet(Biasotti and Houssay, 1932). The objections to this interpretation are that the gluco-neogenesis from fat has never been clearly demonstrated, and that the disturbance in nutrition caused by a high fat diet makes deductions unreliable. Russell (1938a&b) favoured the theory that hypophysectomy removes a factor which inhibits carbohydrate oxidation, so that the animal can once more, to some extent, oxidize carbohydrate. This attitude is supported by the observations that the isolated muscle of Houssay dogs has a relatively high R.Q. (Shorr,Richardson and Sweet,1936) and that a Houssay dog shows an increase in R.Q.after ingestion of sugar(Houssay and Biasotti, 1931). This hypothesis is also supported to some extent

by the study of effects of pituitary extracts on R.Q., B.M.R. and glycogen stores of the normal and hypophysectomized rat (see below).

In the final discussion at the end of this thesis, another alternative will be put forward to explain the Houssay phenomenon (see p. 123).

The Role of the Pituitary in Protein Metabolism.

It has been widely accepted that the pituitary plays a prominent role in gluconeogenesis from protein. The following evidence has been advanced to support this hypothesis. Fasted hypophysectomized dogs excrete less urinary nitrogen than normal controls (Braier, 1931,b). Hypophysectomized-depancreatized animals excrete a relatively small amount of sugar. Such doubly operated animals, when injected with pituitary extracts, show an increased nitrogen and sugar excretion (Long and Lukens, 1936). Finally, if hypophysectomized dogs are phloridzinized, they excrete relatively less sugar than normal animals (Biasotti and Houssay, 1932).

The evidence, although to some extent convincing, is open to other interpretations. The changes in nitrogen excretion described may well be accounted for as secondary

effects to the primary disturbance in metabolism of such operated animals. The fasted hypophysectomized animal is probably burning more carbohydrate which may exert a protein-sparing action. The diminution of nitrogen excretion after hypophysectomy in a depancreatized animal may also be explained on the basis that the originally increased nitrogen excretion was a compensatory phenomenon to maintain the depleted carbohydrate stores by gluconeogenesis from protein. A similar secondary increase in nitrogen excretion may well explain the results of Long and Lukens (1936) with pituitary extracts mentioned above.

Biasotti and Houssay (1932) found that hypophysectomized dogs, when phloridzinized and fasted, excrete not only much less sugar but also less nitrogen than controls and rapidly go into hypoglycaemia. This fact is open to more than one interpretation, depending on whether one stresses protein, fat or carbohydrate metabolism as being the primary source of dysfunction after hypophysectomy. They also found that meat or sugar diet would prevent hypoglycaemia, but that a fat diet was not effective. This fact has been discussed elsewhere in relation to gluconeogenesis from fat. They observed that in the fasted, hypophysectomized dog the nitrogen excretion is decreased; however, thyroidectomy produced a

similar effect. Phloridzin administration plus a meat diet produced a glycosuria only slightly less than normal controls. They concluded that the capacity to form sugar from endogenous but not exogenous protein is diminished in hypophysectomized dogs. This observation agrees with the results of Braier (1931,b) who found that on fasting, hypophysectomized dogs eliminated 40% less nitrogen and creatinine than controls.

Evans (1936) found that rats placed in a low oxygen tension appeared to synthesize and store carbohydrate. The greater nitrogen excretion was sufficient to account for the newly formed carbohydrate. Hypophysectomy or adrenalectomy abolished the effect (Evans, 1934).

It is worthy of special consideration, however, that Aschner (1912) and Braier (1931,b) observed a normal nitrogen excretion in well nourished hypophysectomized dogs. Furthermore, the influence of pituitary extracts on nitrogen excretion gives very little evidence to support the importance of the pituitary in gluconeogenesis from protein. Most observers report that such extracts diminish nitrogen excretion (see p.25). The only exceptions are where a definite hyperthyroidism is produced by the thyrotropic hormone, or where a frank glycosuria ensues, as in depancreatized-hypophysectomized animals (Long and Lukens, 1936, already referred to above).

In conclusion, the published results do not prove a direct influence of the pituitary on protein metabolism, but indicate that under abnormal conditions the animal appears more reluctant to call upon its tissue reserves of protein. This phenomenon may be due to the excessive carbohydrate oxidation, occurring under such conditions as fasting, and thus exerting a protein-sparing action on metabolism.

After hypophysectomy in young animals a complete cessation of growth is observed, and for the following reasons this is generally interpreted as being due to lack of a specific growth-promoting hormone of the anterior pituitary. Extracts of the anterior lobe can induce a renewal of growth, and if the epiphyses do not close, as in rats, gigantism may be produced (Evans and Long, 1921; 1922). The active principle is apparently not identical with the other known hormones of the anterior lobe (Evans, 1938). Furthermore, there is ample clinical confirmation of the relationship between the activity of the anterior lobe and growth, since the early observations of Pierre Marie on gigantism with tumours of the anterior lobe and of Paultauf on dwarfism with atrophy of the anterior lobe of humans.

Since this thesis is not concerned directly with this aspect of the pituitary, further discussion of the growth-promoting hormone and its mode of action is omitted.

The Direct Effects of Pituitary Extracts on Metabolism.

This section concerns the effects of pituitary extracts on normal and hypophysectomized animals which are not due, as far as is known, to a trophic influence on any other endocrine gland. This arbitrary distinction is based on the shorter latent period as well as the production of an effect not involving one of the known dependent glands.

The Influence of Pituitary Extracts on Fat and Carbohydrate Metabolism.

Anselmino and Hoffmann (1931), in two consecutive papers, demonstrated that anterior pituitary extracts produced ketonemia in non-fasted rats on a normal diet. Extracts of posterior lobe were not effective. The active principle was obtained by an aqueous extraction of the acetone-dried powder and filtration through a collodion membrane. They found that it particularly increased the β-hydroxybutyric acid fraction. The peak-point of ketonemia occurred about the second hour. The active principle was destroyed by heating for 15 minutes at 60°C; it was soluble in 50% alcohol, and was sensitive to ultra-violet light. It was adsorbed by kieselgur, but

not by charcoal. Using 1000 mg. of such an extract on a human subject, the oxygen consumption decreased immediately - an effect reminiscent of the effect of vasopressin (Geiling and deLawder, 1932). A fat meal given to humans produced a substance in the serum apparently identical with the pituitary principle. Serum from patients given a carbohydrate or protein meal did not possess this property.

There appears to be very little doubt that they were dealing with a hitherto undescribed active principle. Its properties and distribution bear no resemblance to the melanophore hormone. Their results on rats have been amply confirmed by Magistris (1932) and by Eitel, Löhr and Loeser (1933), and Bonheim and Heiman(1932)

Some modifications of the procedure have been adopted. Burn and Ling (1933) studied the acetonuria in rats on a high fat diet. Others preferred to sensitize the animal by a preliminary fast. Many workers preferred to study the ketonuria rather than the ketonemia (Burn and Ling, 1933; Butts, Cutler and Deuel, 1933-34; and Black, Collip and Thomson, 1934).

The original workers termed the active principle the "Fettstoffwechsel Hormon". Magistris named his preparation "Orophysin". The less speculative term

"ketogenic principle" was adopted in English literature, because its relation to fat metabolism remained problematical. Black, Collip and Thomson (1934) separated the active principle from growth-promoting, thyrotropic and adrenotropic hormones, and showed that continued injections produced resistance. The serum of a horse subjected to prolonged injections inhibited the ketogenic effect of the extract on rats.

Raab (1926; 1934) described a pituitary fraction obtained from the anterior and posterior lobes and hypothalamus, which caused a decrease in blood fat concentration (lipoitrin). It was supposed to act via the tuber cinereum. It increased liver fat and diminished liver glycogen. Blood ketones were only slightly affected (usually diminished). It was heat resistant but sensitive to alkali. This property served to differentiate it from the melanophore-expanding hormone which has a similar distribution. He attributed to it a role in the absorption of fat from the circulation by the liver. He quoted the work of Feuling, to the effect that the R.Q. was not influenced by such extracts.

These findings have never been adequately confirmed. Himwich, Haynes and Spiers (1930), working with dogs, found that pituitrin usually caused a decrease in blood fat, but sometimes both a rise and a fall occurred. Oxytocin and vasopressin had a similar but

less constant depressing effect on blood fat. Munoz (1933) used even 100 mg. per kilogram of standard pituitrin on dogs and found no decrease. Other workers (George, 1930; Long, Hill and Bischoff, 1932), using rabbits, failed to find any significant change. Rony and Ching (1930) could not confirm Raab's observation that posterior lobe extracts lowered alimentary lipaemia of dogs.

In 1927 Johns, O'Mulvenny, Potts and Laughton reported glycosuria in dogs following repeated injections of crude anterior pituitary extracts. Baumann and Marine (1932) and Evans, Meyer, Simpson and Reichert (1932) confirmed the observation on rabbits and dogs respectively. Houssay, Biasotti and Rietti (1932), Evans (1933), Barnes and Regan (1934) obtained marked glycosuria on normal dogs with similar treatment. The "diabetogenic" effect occurred after treatment with relatively large doses for several days, the equivalent of up to 2 gm. of anterior pituitary per kilogram being given per day. The response resembled true diabetes in the rise of blood fats, blood sugar and urinary nitrogen. A high carbohydrate diet apparently facilitated the effect. After a period of some days the response diminished unless large doses were used. Shipner and Soskin (1934) described a similar effect on normal dogs, but it differed from Houssay's results, since it was only a transient glycosuria after

each injection. With prolonged injections the effect passed off (see also Evans, 1933). They considered that the adrenals were essential. However, when given to depancreatized dogs maintained on a constant insulin intake, their extract produced a steadily increasing diabetes over a period of several days. Hence it appears possible that the immediate hyperglycaemic response is the same as the delayed effect described by Houssay, et al (1932).

In Houssay's laboratory it was shown that the R.Q. failed to rise after injection of glucose plus the diabetogenic extract; the animals had a decreased sugar tolerance (Biasotti, 1934) and a marked insulin resistance (di Benedetto, 1933).

Young (1937) and Campbell and Best (1938) have recently reported the persistence of a diabetic state in chronically injected normal dogs after withdrawal of the injections.

Some of the above authors observed a concurrent ketosis with the glycosuria (Rietti, 1934).

Houssay and Biasotti (1931) and Houssay, Biasotti and Rietti (1934) have studied the properties of the diabetogenic principle. They found it to be easily adsorbed on animal charcoal; it is not ultra-filtrable and does not dialyze. Heating for 15 minutes at 80°C. destroys it. It is partially soluble in 50 % alcohol.

In the absence of the thyroid, adrenals and gonads, pituitary extracts elicit a typical diabetogenic response in depancreatized-hypophysectomized toads (Houssay and Biasotti, 1933). However, the liver is considered essential in both the toad (Campos, Curutchet and Lanari, 1933) and the dog (Houssay and Foglia, 1936).

Recent workers, particularly Long (Harvey Lectures, 1936-37) and in this laboratory (Collip, 1934) prefer to use a more sensitive test animal for assay of the diabetogenic effect, the depancreatized-hypophysectomized dog or cat being commonly used. The terminology of the various activities is somewhat confused. The terms "glycosuric" and "diabetogenic" are sometimes used synonymously and do not necessarily indicate the presence of ketosis, while the "ketogenic principle" is used to describe ketonuria and ketonemia alone. There is, to date, little convincing evidence to suppose that these effects are caused by different hormones. The terminology may be merely descriptive of the varying responses of animals in different nutritive states. Thus the fasted animal may be readily ketosed, but a concomitant hyperglycaemia and glycosuria are rare. On the other hand, a depancreatized-hypophysectomized animal may readily show glycosuria with or without ketosis. However, Lucke (1933) has pointed out that his contra-insulin hormone of the anterior pituitary

which was apparently identical with the hyperglycaemic factor, was not ultrafiltrable, and so differed from Anselmino and Hoffmann's Fettstoffwechsel Hormon.

Other workers have suggested that the "diabetogenic" effect is due to a complex mixture of more than one active principle. Young (1936a)suggested that there were three such principles: (a) the ketogenic factor of Anselmino and Hoffmann, which was possibly the same as the factor stimulating liver glycogen formation; (b) a glycotropic factor, which facilitated hepatic glycogenolysis and inhibited to some extent the peripheral action of insulin; (c) a glycogenolytic factor, such as that described by Anselmino and Hoffmann (1935)*. Long (1936-37) favoured the possibility of two fractions in the diabetogenic complex: (a) heat labile,and (b) heat stable. The latter, he found, would produce only glycosuria in doubly operated animals and it required the presence of the former to precipitate ketosis. It is difficult to say whether his results were influenced by dosage. However, it stands as concrete evidence to support the hypothesis of more than one active principle.

To summarize the confusing evidence accumulated regarding the "diabetogenic hormone", it is obvious that the term includes many phenomena. It would be safer to restrict the title to the production of a definite diabetes

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* Extract taken from Young's publication.

as described by Young (1938), Campbell and Best (1938) and to some extent in Houssay's original work. This excludes the phenomena of transient glycosuria in normal or doubly operated animals, and the contra-insulin effect of Lucke. The latter, as will be seen later, is a characteristic of both anterior and posterior lobe extracts, while there is no evidence available that any tissue except the anterior lobe has a diabetogenic or ketogenic action.

Best and Campbell (1936) showed that anterior pituitary extracts injected daily into rats fasted for three days produced a deposition of fat in the liver much greater than in controls (see also Fry, 1937; McKay and Barnes, 1937). Two to three hours after each injection the ketosis reached a maximum. The effect was independent of the thyroid gland, and posterior lobe extracts had a similar but relatively slight effect. Recently the same authors (1938) made a total fat analysis on rats and reported that the injected animals had 6% less fat content than controls. Owing to the fat concentration in liver and kidneys, the depot fat showed an even more significant fall.

Russell and Bennett (1936) undertook a complete carbohydrate analysis of hypophysectomized rats fasted 24 hours, with an without injections of a pituitary "growth" preparation during the fasting period. They found that the extract prevented the abnormal depletion of muscle and liver glycogen previously described by Phillips and Robb (1934) and Russell (1936). In fact the muscle glycogen was elevated to super-normal levels. The active principle was destroyed by boiling for one hour, and the effect was not due apparently to gonadotropic, thyrotropic or mammatropic hormones. It was associated with the growth and adrenotropic fractions.

Young (1936,b) described a glycotropic or anti-insulin fraction of the anterior pituitary which if injected into rabbits during a 22 hour fast produced a remarkable retention of liver glycogen. The active fraction was low in gonadotropic and thyrotropic hormones, but rich in lactogenic activity. He attempted to differentiate between this effect and the "glycostatic" effect of Russell and Bennett since the stores of liver glycogen in his experiments could easily be mobilized by adrenalin or emotional stimulus. His explanation appeared unconvincing and has not yet been supported by published data. As will be seen later, the blood sugar response to

adrenalin given with "posterior lobe" extracts, may be diminished or increased depending on the type of experiment (see p. 28). Young observed that the possibility of gluconeogenesis should be borne in mind in the interpretation of his and Russell and Bennett's results.

Meyer, Wade and Cori (1937), using male rats, 24 hours fasted, gave a known quantity of glucose one and a half to three hours before an injection of a growth preparation of the anterior pituitary. Three hours after the sugar ingestion, the animals were killed and liver and muscle glycogen estimated. The B.M.R. and R.Q. were studied during the last three hours. The extract compared to controls obtained with boiled extracts caused a decrease in R.Q. (.87 to .77). The oxygen consumption was stimulated + 7% and +21% in two groups. The liver and muscle glycogens were both significantly higher in the treated group. The authors discussed the results from the point of view of inhibition of carbohydrate metabolism. They avoided the possibilities of gluconeogenesis from fat (Cathcart and Markowitz, 1927) or an increase in fat oxidation with depression of carbohydrate oxidation, notwithstanding an increased B.M.R. with a lowering of the R.Q. Russell (1938,b) obtained almost identical results on glucose fed normal rats, and favoured a similar interpretation.

An important publication by Fisher, Russell and Cori appeared in 1936. They confirmed the observation of Fisher and Peneharz (1936) that fasted hypophysectomized rats have a higher R.Q. than normal. They treated such fasted animals with anterior pituitary extracts and found that the R.Q. fell to the level of normal fasted rats, and that the abnormal dissipation of liver and muscle glycogen was inhibited. The oxygen consumption showed a surprising drop during the six hours after injection (-14%) from the usually low level of hypophysectomized animals. Apparently, taking the latter fact into account, they concluded that the hypophysectomized animal is deficient in a carbohydrate-depressing mechanism, and that the pituitary extract acted in virtue of its ability to suppress this unrestrained oxidation of carbohydrate. The extract was apparently similar to that used by Meyer, Wade and Cori (1937) and Russell (1938,b) noted above. In fact Russell also noted that the hypophysectomized, glucose-fed rats showed a slight decrease in B.M.R. (-6%) after injection in contradistinction to her results in normal fed animals mentioned above. These observations on the depression of oxygen consumption in hypophysectomized animals are especially worthy of consideration since they form

the basis of one interpretation of pituitary deficiency - i.e., a lack of a carbohydrate inhibitory factor.

The Influence of Pituitary Extracts on Nitrogen Metabolism.

Many investigators have studied the effect of pituitary extracts on nitrogen metabolism with a view to obtaining some biochemical substantiation for the growth-promoting hormone. It is obvious that a true growth stimulation should be associated with a decreased protein catabolism and an increased protein anabolism. The "growth" preparations used were usually impure, and contained appreciable amounts of other pituitary hormones.

With such extracts, Teel and Cushing (1930) observed a fall in blood non-protein nitrogen and a diminished nitrogen excretion in dogs. Gaebler (1933;1935) noted a marked diminution in urinary nitrogen of normal and thyroidectomized dogs during the week following a single large injection (50 c.c.). The blood non-protein nitrogen showed a corresponding diminution. The urea was the factor chiefly involved. From the simultaneous increase in oxygen consumption and the low R.Q. he concluded that fat oxidation was increased. In some cases the retained nitrogen was subsequently lost during the recovery period. Gaebler and Price (1937) studied the different urinary constituents under the same conditions. They pointed out that the changes occurring

in sodium, sulphur and phosphate excretion bore a resemblance to those which occur when a rapid protein synthesis is taking place, such as in convalescence from a wasting disease.

Other workers have corroborated the finding that "growth-promoting" extracts diminish nitrogen excretion (Shaffer and Lee, 1935; Reiss, Schwartz and Fleichmann, 1936; Mirsky and Swadesh, 1938). The latter workers used the rate of increase of blood non-protein nitrogen in the nephrectomized dog as an index.

This review is not concerned with any effects mediated through the thyroid by the thyrotropic hormone. The only other observation in the literature which indicates an increased nitrogen excretion with pituitary extracts comes from workers with the diabetogenic principle. This has been discussed already (see p.10 and p.11).

Some Observations on the Effect of Posterior Lobe Extracts on Metabolism.

In this section an attempt is made to avoid discussion of effects due solely to the posterior lobe active principles oxytocin and vasopressin. It is the object to concentrate on certain effects produced by posterior lobe extracts which closely resemble those produced by similar anterior lobe extracts.

As will be discussed later, posterior lobe extracts have a high melanophore content. This is partly due to inclusion of an appreciable portion of the pars intermedia when the gland is divided. There is in general a striking resemblance between the metabolic effects of extracts from both lobes. In the early years of this century Harvey Cushing (1910) stressed the importance of the posterior lobe secretion in carbohydrate metabolism. A deficiency of its secretion, he postulated, promoted a high sugar tolerance and obesity, while injection of posterior pituitary extracts caused glycogenolysis and hyperglycaemia. Borchardt (1908) produced glycosuria with posterior lobe extracts. Burn (1923) confirmed these findings and showed that such extracts neutralize the hypoglycaemia of insulin.

In the hands of other workers the blood sugar response has been hypoglycaemia. In fact the results of Burn (1923) show a bewildering variation with the same extract in different rabbits. Some show a rise, some no change, and some a rise or no change followed by a decrease. The hypoglycaemia would appear to be due to a secretion of insulin (Blotner and Fitz, 1927; LaBarre, 1927 and 1928, who used the cross-circulation technique). Zunz and LaBarre (1934) also obtained evidence that anterior lobe extracts excite insulin secretion in dogs.

Another peculiarity of posterior lobe extracts is their ability to neutralize the hyperglycaemia of adrenalin (Stenström, 1913; Burn, 1915 and 1923). Possibly this property is due to an increased insulin secretion. However, Houssay and di Benedetto (1933) found that intravenous injection of pituitrin with adrenalin may accentuate the hyperglycaemic action of the latter.

Raab (1930; 1934), from a study of oxytocin, vasopressin and pituitrin on neutral fats and phosphatides of blood, considered "the intermediary and posterior lobe of the pituitary as important factors in the maintenance of normal quantitative conditions of fat balance". His work has already been discussed (see p. 16).

It is obvious from these observations on "posterior lobe extracts" that the posterior lobe and pars intermedia should not be ignored for their metabolic significance. Houssay (1936) has pointed out that in the hypophysectomized toad, extracts of the intermedio-neural lobe compensated for the metabolic and general symptoms of pituitary insufficiency, such as general asthenia, sensitivity to insulin and reappearance of diabetes if the pancreas had been previously removed. Furthermore, only extracts of the principal lobe (the anterior lobe of mammals) had any trophic effects on the secondary atrophy of other endocrine glands. The author is not aware of any evidence in the literature which would suggest that either oxytocin or vasopressin possess properties of such metabolic significance. Holman and Ellsworth (1935) have reviewed the literature on their effect on blood sugar. Ellsworth (1935) considered that the dosage required to produce an effect on blood sugar of rabbits was too high to be considered of physiological importance. Hence it is not incompatible with the evidence to attribute to the pars intermedia the effects produced by posterior lobe extracts.

The Direct Effect of Pituitary Extracts on Gaseous Metabolism.

In addition to the well established influence of the thyrotropic hormone on oxygen consumption, there are a number of interesting observations in the literature on the effect of pituitary extracts on gaseous metabolism. Hoffmann and Anselmino (1931) and Magistris (1932) observed an immediate lowering of the B.M.R. after injection of ketogenic extracts. Black (1935), using a crude alkaline extract, found an immediate and delayed decrease in B.M.R. However, on one guinea pig studied, the B.M.R. and R.Q. increased. The latter change was not considered significant owing to the blowing off of CO_2.

Lee and Gagnon (1930), working with rats, and Teel and Cushing (1930) with dogs, studied the effect of chronic injections of growth extracts on gaseous metabolism. Both groups of workers observed a lowering of the oxygen consumption. Anderson and Collip (1934) also reported a lowering of the B.M.R. after chronic treatment of rats with thyrotropic hormone.

Gaebler (1933; 1935), after a single large injection of an impure growth extract, reported a considerable increase in B.M.R. of normal and thyroidectomized dogs. The R.Q. remained low, less than 0.8,

but if an excess of glucose was given the fall in R.Q. was not so apparent. The extract boiled for 5 minutes was inactive. The author suggested an increase in fat oxidation, but did not discuss other possibilities. Riddle, et al (1936), using a prolactin-growth preparation on normal and thyroidectomized pigeons, obtained a rise in B.M.R. The R.Q. was not studied. In the previous section (see p.23,24) mention was made of the work of Fisher and Pencharz (1936), Fisher, Russell and Cori (1936) and Meyer, Wade and Cori (1936) in which they found that pituitary extracts lowered the R.Q. of normal and hypophysectomized rats, and the latter workers also obtained an increased oxygen consumption in normal rats soon after a single injection. The extract used was a growth preparation. It is of interest to recall the observations of Lucke (1933) and Collip (1935) that the contra-insulin and diabetogenic activities were associated with the growth fraction.

Krogh and Okkels (1933) and Dieffenbach (1933) using rabbits, guinea-pigs and rats, found that anterior pituitary extracts rich in thyrotropic activity increased oxygen consumption to even+60 % within two hours after subcutaneous injection. Krogh and Okkels attributed the effect to a rapid secretion

of the thyroid, since they observed changes in the Golgi apparatus of the thyroid epithelial cells. They did not study the effect on thyroidectomized animals. Similarly, Zunz and LaBarre (1935) claimed that the "thyrotropic hormone" produced an immediate secretion of the thyroid because the fall in blood sugar, noted after injection in dogs, was not observed after thyroidectomy. They confirmed their interpretation by showing that the serum after injection decolourized a muscle extract plus methylene blue more rapidly, compared with serum before injection. Evidence is included in the experimental section of this thesis which indicates that the results of these authors were due to a factor other than the thyrotropic hormone (see pp.73,96).

The injection of posterior pituitary extracts has given rise to very variable results (either an increase, a decrease or no change) even in the hands of individual authors. Chahovitch (1930), Himwich and Haynes (1930-31) and Uyldert (1933) studied the effect on rats. Nitzescu and Gavrilla (1929), Schill and Fernbach (1929), Castex and Schteingart (1930) and Hartl (1933) studied it on humans. A possible reason for the variable results is suggested by the recent work of Geiling and DeLawder (1932) and Grollman and Geiling (1932). Using purified vasopressin on dogs and humans respectively, they found a dramatic fall

in oxygen consumption followed by a variable compensatory rise, so that depending on the time after injection, vasopressin may cause a fall, an increase, or no change.

From the wealth of conflicting data accumulated about the influence of pituitary extracts on gaseous metabolism, certain findings appear to be of undoubted significance: first, that the R.Q. of hypophysectomized fasted rats can be lowered to the level of normal controls (Fisher, Russell and Cori, 1936); second, that the pituitary contains a metabolic stimulant other than the thyrotropic hormone (Gaebler,1935 , and Riddle, et al,1936); and finally, that chronic injections produce a low B.M.R. Numerous observations of an immediate fall in B.M.R. after injection must be considered with caution. The shock and collapse following injection of a toxic extract or the possibility of contamination with vasopressin would suggest an explanation for some of these results. The author observed that an extract rich in prolactin on first injection produced an immediate decrease in oxygen consumption. A second injection the following day actually produced a slight increase. Possibly the animal became adapted to the toxic effect after one injection.

General Discussion and Summary.

In a wide variety of species, soon after hypophysectomy certain deficiency signs become manifest which are apparently not due to a lack of any of the known trophic hormones. The animals exhibit a remarkable tendency to hypoglycaemia, especially if fasted; a hypersensitivity to insulin; and if the pancreas has been previously removed, an alleviation of the cardinal signs of diabetes. Furthermore, in the young animals growth ceases.

If the animal is well nourished the metabolism is balanced on the normal proportion of protein, fat and carbohydrate, as indicated by nitrogen excretion, glycogen stores and the R.Q. However, after a period of fasting, if fatal hypoglycaemia does not ensue, metabolism shows a preponderance towards carbohydrate oxidation, since there is a low nitrogen excretion, a relatively high R.Q. and dissipation of carbohydrate stores.

These phenomena have been interpreted under two widely divergent theories: (a) an inhibition of gluconeogenesis particularly from fat; (b) a lack of an inhibitory influence on carbohydrate oxidation. The evidence advanced in support of each of these hypotheses has been reviewed. The cessation of growth is generally

considered to be due to a lack of a separate specific growth-promoting hormone.

Injection of pituitary extracts has a wide variety of effects on metabolism. The following list summarizes the well recognized responses elicited by various extracts of either the anterior or the posterior lobe.

(1) Hyperglycaemia and glycosuria (immediate and delayed).

(2) Inhibition of insulin hypoglycaemia.

(3) Ketosis.

(4) Deposition of lipoids in the liver.

(5) Depression of R.Q.

(6) Variable effects on B.M.R.,particularly an increase.

(7) Lowering of blood lipoids.

(8) Retention of carbohydrate stores -"glycostatic".

(9) Glycotropic effect on liver glycogen.

(10) Ability to neutralize the hyperglycaemia of adrenalin.

(11) Insulin secretion.

(12) Nitrogen retention.

(13) Promotion of growth in young hypophysectomized animals.

It seems hardly reasonable to suppose that the pituitary gland which is already secreting the known trophic hormones could also be the site of formation of so many metabolic "hormones". It is more likely that many of these effects are due to the same active principle. Considering the impurity of extracts used and the lack of knowledge of their physical and chemical properties, it is inadvisable to speculate on the identity of the various active principles described above.

The Melanophore Hormone.

The pioneer work of Smith (1916) and Allen (1916) provided the first definite indication that the pituitary gland controlled the melanophores of frogs. These authors found that after hypophysectomy the frogs became pale, apparently due to contraction of melanophores. Further evidence to support the role of the pituitary rapidly accumulated. Atwell (1919) and Smith (1920) claimed that extracts of pars intermedia of beef pituitary darkened the silvery hypophysectomized tadpoles. Allen (1920) and Swingle (1921) transplanted the lobes of the frog's pituitary from adult frogs into normal and hypophysectomized tadpoles, and produced marked darkening with pars intermedia. Anterior-lobe grafts produced a temporary darkening, which disappeared although the graft persisted and caused acceleration of growth and metamorphosis (Allen, 1920). The association between the pars intermedia and melanophore controls has been also emphasized by Hogben and Winton (1922) and by Van Dyke (1926) who confirmed the finding that the pars intermedia contains a much greater concentration of melanophore than any other portion of beef pituitary.

The final proof that the pars intermedia elaborates the melanophore hormone is based on the following evidence. Anderson and Haymaker (1935) and Geiling and Lewis (1935) were able to demonstrate the growth of pars intermedia tissue in vitro with formation of the melanophore-dilating hormone.

No other pituitary hormone was obtainable from these cultures. Atwell and Holley (1936), by means of brilliant surgical technique, removed the caudal portion of the epithelial hypophysis of toads at a certain stage of development. The tadpoles metamorphosed normally, the adrenals, thyroid and gonads developed as usual, but they assumed the silvery colouring of hypophysectomized frogs. Presumably the anlage of the pars intermedia was removed, leaving the posterior lobe intact and enough of the anterior lobe for normal growth and development. The results were checked by serial section. Finally, Fisher (1937), by cutting the supra-optico-hypophyseal tract in the anterior part of the hypothalamus of cats, produced atrophy of the posterior lobe and stalk. The pituitary glands of such cats, although containing no vasopressin, oxytocin or anti-diuretic activity, retained the ability to dilate the melanophore of frogs.

Further confirmation was supplied by the following workers. Bayer (1930) examined the pituitary of a single unusually pale frog by serial section and found that the pars intermedia had been destroyed by an encysted parasite. In certain species the pars intermedia does not exist as an anatomical entity. In such cases the anterior lobe contains the active fraction. De Lawder, Tarr and Geiling (1934) pointed this out in chicks. Valsö (1934) and Geiling (1935), working on whale pituitary - in which species the anterior and posterior lobes are separate - found that the former lobe contained melanophore hormone,

but no oxytocin or vasopressin. The posterior lobe contained only the latter two hormones. Roth (1932) and Jores and Glogner (1933) investigated the problem on human pituitaries, and found the melanophore present in the anterior lobe, apparently associated with the basophilic cells. A basophilic adenoma of the anterior lobe was particularly rich in the hormone.

The author could find only one observation in the literature which suggests that the melanophore hormone is not formed by the pars intermedia. Jores and Will (1934) studied its concentration in various parts of the pituitary gland and found the maximum concentration designated as 100 % in basophilic cells of ox pituitary. The pars intermedia contained 85 %, the anterior lobe 38 % and the posterior lobe 25 %. Studying the concentrations of the erythrophore hormone, they found different values: 20 % for basophilic cells; 100 % for pars intermedia; 80 % for anterior lobe and 60 % for posterior lobe. However, the difference between 100 % and 85 % is hardly significant considering the methods of assay available. Furthermore, Jores (1936b) ignored his previous suggestion and referred to the melanophore hormone as formed in the pars intermedia.

That the melanophore hormone is a separate entity is now generally accepted. Herring (1914-15) had long before its discovery shown that the posterior lobe had a higher concentration of oxytocin and vasopressin than the pars intermedia. In view of the early work on the melanophore hormone, this should have suggested the probability of its separate identity. However, it was not until recent years that convincing evidence was forthcoming. Dreyer and Clark (1923), by ultra-filtration of posterior lobe extract, found oxytocin and vasopressin in the filtrate, but only a small fraction of the melanophore hormone passed through. Fenn (1924-25) succeeded in a partial separation of three active fractions by means of butyl alcohol extraction followed by aqueous extraction of the residue. But even in 1926 Krogh, after differentiating melanophore hormone from oxytocin by its susceptibility to acid at pH 4.5 to 2.5, spoke of different activities being due to separate atomic groups in the same hormone molecule.

Rowe (1928) supplied the first convincing evidence by a study of the oxytocic and pressor preparations of Kamm, et al (1928). Both preparations, but particularly oxytocin, had relatively low melanophore activity compared to the ratio of the three constituents in pituitrin. Hogben and Gordon (1930) discovered that

melanophore hormone was relatively resistant to alkali. Zondek and Krohn (1932) used this procedure in the final stage of preparing a concentrated melanophore extract from posterior lobe. They discarded the frog as an unreliable means of assay and used a minnow (Phoxinus laevis); the minimal amount capable of producing the "wedding-dress" effect was defined as a unit of "intermedin", as they termed their active principle.

Dietel (1934) was the first to obtain by chemical means a melanophore preparation free of oxytocin and vasopressin from posterior lobe extracts. Stehle (1936) obtained a similar extract which contained traces of the posterior lobe hormones.

The final differentiation of the melanophore hormone depends on the tissue-culture work of Anderson and Haymaker (1935) and Geiling and Lewis (1935), the neurosurgery of Fisher (1937) mentioned above (see p. 38), the preparation of oxytocin and vasopressin free of melanophore-dilating action (Stehle, 1933), and the observation of Geiling (1935) that in the fin-back and sperm whale the melanophore hormone is found in the anterior lobe while oxytocin and vasopressin are found in the anatomically separate posterior lobe. Fraser (1937) has differentiated the melanophore-expanding hormone from the anti-diuretic principle by a study of Stehle's melanophore preparation.

Physical and Chemical Properties of the Melanophore Hormone.

The melanophore-dilating hormone is insoluble in acetone, ether and chloroform. It is easily soluble in water and fairly soluble in high concentrations of ethyl alcohol, particularly if boiled (Jores, 1933,a). Charcoal adsorption is considered by Jores and Velde (1933) as an essential characteristic. However, Dietel (1933,c) found that it was adsorbed with some difficulty. Jores (1933,a) also stressed its destruction by bright light or ultra-violet light, particularly in acid solution.

Hogben and Winton (1922) first described the resistance of the melanophore hormone to pepsin, and its destruction by trypsin (confirmed by Houssay and Unger,1926). The former workers and Jores (1933,a) also observed that it was easily destroyed by hydrogen peroxide, and Jores obtained a similar result with $KMnO_4$.

Dreyer and Clark (1924) and Dietel (1933,c) stated that the melanophore hormone dialyzed with difficulty compared to the posterior lobe hormones. Zondek and Krohn (1932,c) found that their preparation of intermedin dialyzed more readily than oxytocin and vasopressin. It is difficult to compare work of different authors on dialysis owing to variations in technique, in membranes used, and in the degree of purity of the solution used for dialysis. If excess of protein is present one would expect adsorption of the hormone to inhibit dialysis.

Hogben and Gordon (1930) first drew attention to its resistance to alkali. They observed an increased activity after such treatment and attributed it to destruction of the vasopressor principle. Jores and Lenssen (1933) considered that there was also a chemical change in the hormone itself. In 1934 Jores and Will considered that alkali treatment converted a precursor substance into the melanophore hormone, and that blood had in vitro a similar activating influence on the hypothetical precursor. A similar activation by blood has been described by Popa and Fielding (1933). Stehle (1936) suggested that boiling in N/10 NaOH for 15 minutes produced a qualitative change in the hormone, giving rise to a more prolonged but less intense action.

The common standard procedure for separating the melanophore hormone from desiccated pituitaries is to boil in 0.25 % acetic acid for a few minutes, centrifuge and discard the insoluble fraction. Since its resistance to alkali has become known, methods based on this property have been used. Jores and Glogner (1933) used boiling N/10 NaOH and claimed a higher yield. Dietel (1934) used a saturated solution of barium hydroxide in the initial stage, followed by acetone precipitation and boiling absolute alcohol extraction. Finally taken to dryness, the powder is dissolved in water and the residue discarded. Stehle's procedure will be described in detail later (see p. 79).

Methods of Assay of the Melanophore-Expanding Hormone.

The frog's melanophores have been exclusively used as a test object for the melanophore-expanding hormone. Most workers use the intact frog and inject the solution to be assayed into the dorsal lymph sac. The amount equivalent to 0.5 mg. of standard posterior pituitary powder is generally taken as the standard unit. If the following precautions are taken, it is considered reliable. The needle must be of fine bore and not more than 0.5 c.c. volume should be used, so that the injected fluid will not leak out (Dietel, 1932). The pH of the solution should be slightly on the alkaline side of neutrality, as acid solutions tend to give a less marked response. The solution should be isotonic, as hypo- or hyper-tonic solutions may give nonspecific reactions. The sensitivity of frogs has a seasonal variation, being highest in spring and autumn and lowest in summer and winter (Jores, 1933,a).

However, the literature contains numerous references to nonspecific responses in frogs with various drugs. Hogben and Winton (1922) found that nicotine and apocodeine in paralytic doses dilated the melanophores. Collin and Drouet (1935) claimed that most gland and tissue extracts give a positive but weak reaction.

Shen (1937) found that strychnine, chloroform, ether and amyl nitrite elicited positive reactions in both normal and hypophysectomized frogs. Di Mattei (1927) also stated that even preservatives in hormone solutions could produce a positive reaction. Other compounds mentioned in the literature which may in relatively high concentrations dilate the melanophores are quinine, choline, acetylcholine, paraldehyde and caffein, but the effect is variable and even in the hands of other workers acetylcholine has more of a constricting effect (Kobayashi, 1928).

In spite of all precautions there are many contradictions in the literature about the distribution of the melanophore hormone in the body, which are only explicable by the frequency of nonspecific reactions. Dietel (1931) claimed that it was present in various organs and body fluids in small amounts. However, Jores and Velde (1933b) held that such results were due to technical errors in assay and to nonspecific reactions which could be avoided by investigating the physical and chemical properties of the active principle. If a tissue extract gives a positive result, the active principle should also have the physical and chemical properties of the pituitary melanophore hormone

(adsorbed by charcoal, soluble in 70-90% alcohol, destroyed by ultra-violet light in acid solution, resistant to or potentiated by alkali, etc.).

In order to obtain a more accurate means of assay, others have devised various alterations in the technique. Jores (1932) preferred to use pieces of isolated frog's skin (the Trendelenberg method, 1926). This method facilitates numerous estimations at the same time, and changes in the melanophore cells can be checked by microscopic examination. However, there may be even up to 25% error by this procedure. Krogh (1926) used the perfused hind limbs of the frog. It is difficult to see the advantage of this tedious technique if one is only studying the melanophore-dilating effect of pituitary extracts. Hogben and Winton (1922) used the dorsal lymph-sac method, but confirmed the results by microscopic examination of the melanophores. Hogben and Slome (1931) and Spurrell and Raza (1937) used the degree of expansion of the melanophores in the frog's webb. Intraperitoneal injection may be used to avoid the danger of the injected fluid leaking out (McLean, 1928), as may occur from the dorsal lymph-sac. Konsuloff (1934) has favoured the hypophysectomized frog as a more sensitive and reliable test object. However, such variations in technique as described are hardly necessary when one is studying pituitary extracts, since in the dilutions used it is unlikely that nonspecific reactions could occur(Dietel, 1932).

The Question of More than One Chromatophore Hormone.

When in 1932 (a) Zondek and Krohn described the isolation of a chromatophore hormone from the pituitary gland which they assayed on the erythrophores of the fish Phoxinus laevis and called "intermedin", it was generally understood that they were dealing with the melanophore hormone of previous workers. It had practically all the physical and chemical properties of the melanophore hormone except possibly that it was almost completely destroyed by pepsin. It had the same anatomical distribution in the various parts of the gland as had the melanophore hormone (1932, b and c). Since then, however, evidence is slowly accumulating that the intermedin of Zondek and Krohn is not identical with the melanophore-dilating hormone. The evidence for such a separation is based on the following points. Jores and Lenssen (1933) reported that the melanophore fraction is potentiated by boiling in alkali while the erythrophore fraction is destroyed. Also various extracts contain varying proportions of the two hormones (see Collin and Drouet,1933). Jores and Will (1934) pointed out that adrenalin will neutralize the melanophore but not the erythrophore effect. It is stated that acid will potentiate the erythrophore hormone but has no effect, or causes destruction of the

melanophore hormone. They stressed the greater solubility of the erythrophore hormone in alcohol. They found that various parts of the pituitary contain different relative concentrations of the two hormones, but the differences are actually negligible considering the crude methods of assay available. By boiling the desiccated glands in 0.6% NaCl, they obtained an extract containing ten times more erythrophore hormone than melanophore. By using N/10 extraction they obtained twice as much melanophore hormone as erythrophore. Acetic acid extract yielded five times as much erythrophore hormone as melanophore. They quoted another publication (Jores and Hotop) as showing that in different species the relative concentration of the two hormones varied. They suggested the presence of a chromatophore hormone precursor which could be altered by acid or alkali to either form. Jores and Beck (1934) pointed out that the melanophore hormone increased the size of the adrenals of rats, guinea pigs and rabbits (confirmed by Holmquist, 1934), whilst injection of the erythrophore had no such effect. Rodewald (1935) found that pituitaries of frogs kept in darkness were devoid of melanophore-dilating properties although still able to dilate the erythrophores of fish.

Jores and Velde (1933) suggested that as the erythrophore principle is present only in the pituitary and the base of the brain, it is the central form of the hormone, while the melanophore principle is also present in the blood and eyes and therefore represents the peripheral form. The possibility that one can be transformed into the other is also tacitly indicated by the results of Jores and Will (1934) on activation of the melanophore hormone by blood. During this activation the erythrophore activity is diminished.

Zondek and Krohn (1932,c), discussing the possibility that intermedin and the melanophore hormone may not be identical, point out that the dilatation of melanophores is produced by a light stimulus, whilst the expansion of erythrophores of Elritze is associated with spawning. They quote the observation of Lutzberger that the anti-diuretic effect in diabetes insipidus could be produced only by preparations rich in intermedin and weak in melanophore activity. The fractions very active on the frog were inactive clinically.

In conclusion one must admit that the evidence is very strongly in favour of the existence of two chromatophore hormones. However, it is desirable that this hypothesis should be more universally verified. The work hitherto has been confined almost entirely to Germany,

since suitable fish have not been available elsewhere. Young (1934) claimed that the red-bellied Ohio dace was a suitable test object. However, Fisher (1937) found that injection of neutral solutions, distilled water, and even air gave definite reactions.

The Role of the Melanophore Hormone in Mammals.

Attempts to discover the physiological significance of the melanophore-dilating hormone in mammals have not been attended with complete success. The methods of attacking this problem have been varied and the results have often been of dubious value due to the use of impure extracts.

It was natural to associate the hormone with pigmentation, but Jores (1933,b) found its concentration in the pituitary had no relation to the degree of pigmentation or colour of the hair. Zondek (1935) showed that the intermedin concentration of pituitaries from negroes and patients suffering from a wide variety of diseases gave no significant variations from the normal.

Its association with the adrenal cortex was suggested by the high concentration of the hormone in basophilic tumours(Jores and Beck, 1934) which are not infrequently associated with adreno-cortical hypertrophy, and also by the clinical association between pigmentation

and atrophy of the cortex. However, it would appear that the adrenotropic hormone of the pituitary is a separate entity from the melanophore hormone (see p.112). Holmquist (1934) and Jores and Beck (1934) reported that repeated injection of melanophore hormone caused a hypertrophy of the adrenal cortex in rats, guinea pigs and rabbits. It is difficult to say how much of the effect may be due to toxic products in such extracts. The ascorbic acid content of such enlarged adrenals was not affected, but Jores and Beck stated that the adrenalin content was increased. Zondek and Krohn (1932,c), using their preparation of intermedin on rabbits, which had presumably also some melanophore activity, found a slight decrease or no change in the adrenalin content.

Jores (1933,c) suggested a possible relationship between the melanophore hormone and light adaptation. He claimed that instilling the hormone into the human conjunctival sac shortened the adaptation to darkness. Furthermore, it was found that rabbits kept in the dark or with eyes closed surgically have an increased melanophore content of blood, and aqueous humour. The hormone content of the pituitary glands of chickens, humans, guinea pigs and cats was proportional to their visual acuity in the dark. Buschke (1934) could not find any

effect on adaptation to darkness after instilling or injecting the melanophore hormone sub-conjunctivally in humans.

The relationship of the melanophore hormone to the eye was also indicated by Jores and Velde (1933) who claimed that this hormone was not present in any tissue except the blood, the pituitary, the base of the brain and the eye, if one carefully excluded nonspecific frog reactions by a study of the chemical and physical properties (adsorption by charcoal, destroyed by bright light and trypsin, and solubility in 70 to 90 % alcohol). Another fact suggesting the relationship with light was the observation by Koller and Rodewald (1933) that the melanophore hormone was absent from pituitaries of frogs kept in the dark for 3 to 20 minutes.

Jores (1935,b) discovered that intra-cerebral and intravenous injection of alkaline extracts of posterior lobe of the pituitary into rabbits caused a decrease in body temperature and an increase in blood sugar. He suggested that the melanophore hormone acted on the para-sympathetic system, and was an adrenalin antagonist. The suggestion of antagonism to adrenalin had long been known in the case of the frog's melanophores. Hogben and Winton (1922) referred to the work of Corona (1898) and Lieben (1906), who showed that adrenalin contracted the

frog's melanophores. The possibility that this antagonism may be a more general phenomenon is indicated by the findings of Jores and Caesar (1935) that the two hormones exert an antagonism on the migration of the frog's retinal pigment. Jores (1936,b) injected adrenalin into rats and assayed the melanophore content of their pituitaries during the following hour. Following the injection there was a sharp drop in the melanophore content of both the pituitary and the blood. Cortical extracts had a similar effect, but the decrease was more prolonged. Adrenalin after numerous injections appeared to induce hypertrophy of the pars intermedia.

The melanophore reaction of blood and urine of humans has been studied extensively with a hope of obtaining a clue to its function in mammals. There appears to be no doubt that urines, particularly from females, will give a positive reaction. Whether this effect is due to the pituitary hormone or to some non-specific reaction has been discussed. Positive reactions have been reported in a wide variety of clinical conditions. Collin and Drouet (1933) found marked reactions with urines from patients suffering from pituitary tumours, hyperthyroidism and retinal haemorrhages with chloride retention. Drouet, Mathieu and Colleson (1933) found

it in a patient suffering from migraine. Konsuloff (1934) obtained it in nine pregnancy urines. Parhon, Blinov and Branower (1935) found a very marked reaction with urine of oedematous patients (nephritis and cardiac failure). Urine of nine normal controls gave a negative response.

Jores (1936a)studied the physical and chemical properties of the urinary principle which promoted melanophore expansion. He came to the conclusion that it was not the same as that found in the pituitary. His opinion was based on its response to irradiation by ultra-violet light and charcoal adsorption, and the fact that injection of the melanophore hormone did not necessarily produce a positive urine reaction. Raza and Spurrell (1937) investigated the urinary principle and obtained positive results in 98 % of 46 pregnant cases; 41 % of males and 33 % of non-pregnant females gave a plus reaction. They concluded from a study of the reactions of the urinary principle to charcoal adsorption, tryptic digestion, boiling in acids and alkalies, that it was similar to the pituitary principle.

Ehrhardt (1932) studied the melanophore content of blood and urine after injection of the hormone. It disappeared from the blood of animals four hours after injection, and was excreted in the urine during the 24 hour period after injection. A trace was found during the

second 24 hours. In pregnancy the melanophore content of the pituitary of man and animals was not increased. Jores and Helbron (1933) assayed the melanophore content of blood from females - pregnant, in labour and in the puerperium. No change was observed. They concluded that the hormone had no relationship to pregnancy.

Astwood and Geschickter (1936) published results which appeared to prove that blood from a patient with melanosarcoma gave a positive erythrophore reaction. However, they subsequently withdrew their claims (Lewis, Lee and Astwood, 1937) as the result was due to a technical error. They also investigated five other such cases with negative results.

Rodewald (1936) claimed that the blood of cancerous patients inactivated the melanophore-expanding hormone (the Trendelenberg-Kaufmann reaction) in 105 cases out of 109, and in only one out of 25 normal controls. Unfortunately, controls with other cachectic diseases were not performed. In a later communication (1937) she stated that this inactivation, which needs one hour of contact, is due to a specific anti-hormone to the melanophore-expanding hormone. Oestrin had similar properties but was not identical with the anti-hormone. It is of interest that various authors have

described an inhibitory action of pregnancy serum on the melanophore reaction (Trendelenberg and Kaufmann, 1926; Küstner and Biehle, 1927, 1928; Dietel, 1933b). The higher concentrations of oestrogenic substances in pregnancy sera may have influenced their results.

There is a dearth of evidence in the literature on the effect of removal of the pars intermedia in mammals. The technical difficulties involved have outruled this method of procedure in attacking the problem of the function of this tissue. However, the observations of Pencharz, Cori and Russell (1936) are worthy of note. They claimed that they could remove the anterior lobe and leave the pars intermedia and posterior lobe intact (22 rats). Such partially hypophysectomized animals developed the typical hypersensitivity to insulin found in completely hypophysectomized animals, whereas removal of the posterior lobe and pars intermedia with a variable amount of anterior lobe (13 animals) did not produce the hypersensitivity to insulin. However, their results are not in agreement with those of Geiling, Campbell and Ishikawa (1927) who stated that removal of the anterior lobe did not render the dog hypersensitive to insulin, while operative trauma to the posterior lobe did produce such an effect. Chaikoff, Reichert, Larson

and Mathes (1935) also observed that surgical manipulation involving the stalk of the pituitary caused hypersensitivity to insulin in dogs, although the pituitary gland remained intact.

In conclusion, the available evidence does not prove that the pars intermedia possesses any definite function in mammalian physiology.

EXPERIMENTAL SECTION.

Introduction.

This work started originally with the object of studying the thyrotropic hormone of the anterior pituitary in detail on rabbits' metabolism, particularly with the view to finding out the latent period of this hormone, and to study the phenomenon of thyrotropic resistance with large and small doses. The rabbit was chosen since one could study general metabolism by the Haldane method over relatively short periods, and the animal was sufficiently big to allow of bleeding at intervals for assay of the anti-thyrotropic substance. In some preliminary experiments, using crude alkaline extracts of anterior lobe (Burn's) it was observed that the oxygen consumption was stimulated almost immediately after injection (3 hours). This rather interesting phenomenon was considered of greater interest than the problem on hand. If the effect was mediated by the thyroid gland, one would have to revise the general conception of the latent period of the thyroid hormone. If not mediated by the thyroid gland, it suggested the presence of two metabolic stimulants in the pituitary gland.

After identifying the immediate metabolic stimulant as a separate entity from the thyrotropic hormone and as closely related to or identical with the melanophore hormone, the work was directed towards investigating its function in mammals. For this purpose the respiratory quotient of treated animals was studied in detail.

Technique.

The major portion of this work is devoted to estimation of oxygen consumption of rabbits. The animals, except for a few initial experiments, were Chinchilla breed, weighing 2 to 2.6 kilograms. They were obtained from a single source, but were by no means a pure bred stock. This particular breed was chosen for its relatively low and constant B.M.R. Both sexes were used, but females were more adaptable to training and gave more consistent results. The diet consisted of rolled oats and alfalfa, with fresh vegetables once or twice a week. The animals were kept at least one month in the laboratory, preferably in single cages, before inclusion in an experiment. During this time they were accustomed to the requisite manipulations for estimation of oxygen consumption. Rabbits showing any signs of disease, such as loss of weight, and particularly otitis media, were discarded.

The oxygen consumption was estimated by a modified open Haldane method (1892) in a constant temperature room, over periods of at least one hour. If the R.Q.was also studied the period was extended to two hours. The technique was essentially similar to that already used in this laboratory by the late Dr. Peter Black, and by Dr. Billingsley, to whom the author is much indebted. The apparatus as described by Dr. Black (Ph.D Thesis, 1935) was simplified to some extent as follows. Since the incoming air pressure is constant and the

suction-pump vacuum is constant, it is only necessary to fix the former at an approximate level and subsequently adjust the latter by thumb-screw until the air in the animal jar is at atmospheric pressure, as indicated by the mercury manometer. The arrangement of absorption bottles was also slightly altered. The incoming air was first passed through concentrated H_2SO_4 to remove all water, and subsequently through NaOH flakes, and another bottle of H_2SO_4 to prevent NaOH dust blowing over into the animal jar. The out-going absorption train was passed through: (a) H_2SO_4 concentrated; (b) calcium chloride, and three bottles of NaOH flakes (c, d and e).

Certain difficulties arise in getting an accurate R.Q. with this arrangement. If the following precautions are observed, the apparatus should be suitable: first, the calcium chloride should be almost dust free. This is obtained by blowing dry air through it for a short time before using a recently filled bottle. If the H_2SO_4 in bottle (a) is frequently refilled (about once a week), and if the inlet tube breaks up the air into fine bubbles, the calcium chloride bottle (b) should last indefinitely. The NaOH flakes are more troublesome. They also should be relatively dust free. The first bottle (c) should be turned so that its outlet tubing is at the bottom. This gives a more perfect absorption. The bottles (d) and (e) should be turned so that the outlet is above. This provides an air-lock for fine dust particles.

The amount of CO_2 absorbed in two hours in bottle (d) should not be greater than 10 % of that absorbed in bottle (c). This is a reliable criterion of when to refill bottle (c).

Between each estimation, the bottles of NaOH flakes should be carefully shaken to ensure proper mixing and to prevent pathways forming. Bottles should be weighed only when they have returned to approximately room temperature. The error in weighing two hours after use is negligible.

The use of concentrated solution of NaOH is not recommended, as in a rapidly flowing air current water is given off and must be reabsorbed by H_2SO_4. Thus it adds greatly to the pressure which must be overcome, and correspondingly increases the possibilities of leaks at the rubber junctions.

The apparatus should be frequently tested under pressure for leaks. Defective rubber tubing is the commonest source of error in this respect.

A major source of error occurs in weighing the animal. Two minutes is the maximum time which should be allowed; if any longer time is taken, respiratory distress occurs, causing movement and panting. If a weighing is not accomplished in two or three minutes, it is better to return the animal to the air circuit, and try another weighing ten minutes later.

If the animal urinates during a metabolism, the R.Q. may be unreliable owing to panting. However, personal

judgment should be allowed in each case. If the animal turns in the metabolism jar, it makes the final weighing difficult unless it corrects its position on standing the jar vertical. Finally, before a metabolism reading is begun, the animal should be placed in the jar, disconnected from the second absorption train, for 10 to 15 minutes, until temperature equilibrium is established.

Where a constant record of gaseous metabolism is required in two-hour periods over a total of 8 hours, two absorption trains are used alternately.

The apparatus was checked by combustion of ethyl alcohol under conditions, as regards time and rate of oxygen consumption, which were almost identical with the actual experiments. A small crucible, covered by tin foil and sealed by plasticine, was used. The tin foil was pierced by a fine capillary tubing containing a wick. The alcohol burnt slowly over a period of some hours. An average of ten consecutive experiments yielded .675, or .008 above the theoretical value (max.=.680, min.=.664).

In initial experiments the animals were fasted 24 hours, but to facilitate frequent estimations on a single animal, this point had to be disregarded, and the only precaution taken was to avoid a large food intake during the previous twelve hours. Hence the term "basal metabolic rate" should be avoided in preference to "resting oxygen consumption". Fortunately, as will be seen later, the nutritive state of the animal matters little in the present problem.

Rabbits store food in the alimentary tract for such a long time that specifying the exact number of hours fasted means little. The best indication of the nutritive state is the R.Q. (before injection). This point should be borne in mind in interpreting results.

The procedure adopted to study the immediate effects on metabolism was as follows: after a control estimation of resting oxygen consumption (referred to as the pre-injection level) the extract was injected and a varying number of estimations were taken during the following 4 to 8 hours. No food was given during this period. The data are expressed here as litres of oxygen per square metre of body surface per 24 hours, using Meeh's formula, and Rubner's K = 12.5 (Lusk, 1928). The mid-point of each estimation is recorded as the time after injection, but where the R.Q. was also studied the two hour interval is recorded on tables as such. In certain cases, in order to condense results, the highest point in the post-injection curve is expressed as a percentage change from the pre-injection level. It was found advisable to study the complete curve over some hours after injection, owing to the varying rate of absorption of different extracts. Thus Burn's alkaline extract of sheep anterior pituitary may not attain a peak until the seventh hour, whilst Burn's extract from ox or the purified preparations described later usually attain the maximum effect at the third to the fourth hours. (See Fig. II for typical examples of each).

Most of the work concerning assays and the study of the chemical and physical properties of the active principle to be described, was carried out on chronically injected animals. The injections were spaced at approximately twice per week to allow a sufficient recovery period. This method has the advantage of using an animal of known sensitivity. If for any reason an animal became refractory, it was discarded.

Melanophore-dilating activity of extracts was assayed on the intact frog. The volume injected into the dorsal lymph sac was 0.5 c.c. The required dilutions were made with saline, and the pH was adjusted to the alkaline side of neutrality. Saline controls were included in each group.*

Blood sugars were estimated by the method of Hagedorn and Jensen (1923). Liver glycogens were estimated by the method of Good, et al (1933) . The operative technique was similar to that used in this laboratory by Bachman and Toby (1936). The CO_2 combining power of plasma was done by the method of Van Slyke and Cullen (1917), and the blood urea concentration according to Van Slyke and Cullen (1916). Urinary nitrogen excretion was determined by the micro-Kjeldahl method.

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* The minimal amount which produces a well-marked darkening of the back was used as a frog unit.

TABLE I.

IMMEDIATE INFLUENCE OF BURN'S EXTRACT OF ANTERIOR LOBE ON B.M.R.

Dose = 5 c.c. (1 gm. of original gland) intraperitoneally.

Serial No.	Sex	Species of Gland	Max. % Increase in B.M.R.	Time in hrs. at which peak occurred
7		sheep	+ 25	7 th
8		"	collapse after injection	-
9		"	+ 13	6 th
30		ox	+ 44	3rd to 8th
31		"	+ 28	3rd to 5th
32		"	no change	-
33		"	" "	-

Details of Pituitary Extracts Used.

The first extract used was a simple N/5) alkaline extract of dissected anterior lobe (Burn and Ling,1933). This method gives a crude solution containing all the well established anterior pituitary hormones. Although the results with this extract usually gave a definite increase in metabolism immediately after injection, it was not found very suitable for general use, since it had to be injected intraperitoneally, and the large amount of protein present probably influenced B.M.R. In one case it was definitely toxic, causing an almost instantaneous fall in B.M.R with collapse of the animal. (see Table 1).

The second type of extract used was the concentrated thyrotropic solution (Collip, 1934). This contains variable amounts of gonadotropic hormone, depending on the species from which the glands were obtained. It is essentially a 70 % alcohol precipitate.

The third type of extract used was designated"A.I.F." It was prepared by Doctor Collip by concentrating to an aqueous phase the alcoholic filtrate obtained in precipitating the thyrotropic and gonadotropic substances. This solution was saturated with ammonium sulphate and the precipitate removed. This was taken up in water, washed with ether, and again precipitated with ammonium sulphate. The aqueous solution of the precipitate was made up to 90 % alcohol with absolute

alcohol and the resultant precipitate removed, dissolved in water and again precipitated by 90 % alcohol. The aqueous solution of this precipitate was precipitated isoelectrically and the filtrate was found to be rich in the metabolic factor. This solution contained 1.13 % total solids, including 0.5 % $(NH_4)_2 SO_4$.

"Poly-B" extracts were made by simple aqueous extraction at pH 5 of the acetone dried glands. If this extract was concentrated further, it was termed " 622 ".

The other extracts used are described in the text as they occur with reference to the original publication on their preparation.

Control Experiments, with Other Tissue Extracts.

To study the variation in metabolism from hour to hour, as well as the disturbing influence due to injection, 10 rabbits each received 4 c.c. of water (the same volume as that of extract used) subcutaneously after an initial record of oxygen consumption. During the following 5 hours, a total of 34 records of metabolism were obtained and gave the following analysis when expressed as percentage change from pre-injection level: Aver. = 103.7%; Max. + 12 %; Min. -8 %; Standard deviation = 4.7. Further, the effect of crude alkaline extracts made from kidney, liver and muscle, were studied on four rabbits not used in the above group. Each animal received 5 c.c. of each extract, equivalent to

TABLE 2.

EFFECT OF ALKALINE EXTRACT OF PINEAL GLAND ON GASEOUS METABOLISM.

Serial	Sex	Dose and Extract	Time hrs.	% Change in O_2 consumption	R.Q. Before	R.Q. After
51	F	5 c.c.Pineal Extract	2 - 4	+ 12	.93	.91
51	F	5 c.c.Burns Extract	2 - 4	+35	.80	.74
Control		of Pituitary	6 - 8	+24		.71
54	M	5 c.c.Pineal Extract	2 - 4	+ 7	.74	.71
54	M	5 c.c.Burns Extract	2 - 4	+23	.93	.83
Control		of Pituitary	6 - 8	+27		.80

TABLE 3.

EFFECT OF PANCREATIC EXTRACT 3 * ON GASEOUS METABOLISM.

Boiled at pH 10 for 15 mins.

Serial No.	Sex	Dose c.c.	O_2 Consumption before injection	Time in hours	% Change in O_2 consumption	R.Q. Before	R.Q. After
51	F	15	132	2 - 4	+ 9	.89	.90
52	F	15	100	2 - 4	+ 9	.87	.91
53	F	15	91	2 - 4	+25	.81	.82
54	M	15	113	2 - 4	+26	.83	.85
				Average	+17.2	.850	.870

* An acid alcohol extract of pancreas.

1 gram of tissue, intraperitoneally on three consecutive days. Analysis of 46 post-injection results showed an average of 99.6% (standard deviation = 7.1). In no case was there a typical curve as found with pituitary extracts.

Further controls were obtained with Burn's extract of pineal gland, in which both the R.Q. and oxygen consumption were studied (see Table 2). The increase of + 7% and +12% are within the limits of the previous data on liver, kidney and muscle. The R.Q. shows a slight decrease, but is hardly reliable owing to the impurity of the extract. Table 3 is a summary of controls done with a pancreatic extract. This is the only case where a non-specific tissue extract caused a significant rise in oxygen consumption. However, the R.Q. was not changed. Possibly the increased metabolism may be due to some irritant in the extract as the animals were somewhat restless following injection.

The Immediate Metabolic Increase with Pituitary Extracts, on Normal and Thyroidectomized Rabbits.

Table 4 shows the immediate effect of a thyrotropic extract (A) on oxygen consumption. Table 5 gives the results with the same extract used on 9 thyroidectomized rabbits within 8 days after operation. The response is less in the latter group, but the difference is not considered significant, owing to wide individual variation; also, R.31 had been tested one month before operation with a similar extract and had shown the same response, and R. 26 had

TABLE 4.

NORMAL RABBITS: 4 c.c. PITUITARY EXTRACT "A"

	Serial No.	Sex	Aver. of Controls*	Pre-Inj. Level*	Post-Inj. Max. Rise %
Intraperitoneal -	11	F	127	122	+ 38
"	12	M	143	151	+ 25
"	13	M	143	137	+ 17
"	14	M	131	134	+ 20
"	15	F	119	122	+ 42
Subcutaneous -	18	M	141	141	+ 21
"	19	M	144	141	+ 28
"	16	F	129	125	+ 35
"	21	F	138	129	+ 47
				Aver.....	+ 30

* Litres of oxygen/squ.metre body surface/24 hrs.

TABLE 5.

THE EFFECT OF THE THERMOSTABLE METABOLIC STIMULANT ON THYROIDECTOMIZED RABBITS.

	Serial No.	Sex	% Rise with inj. within 1 wk.	% Rise after 3-6 wks.	Residual Thyroid mg.	% Fall in B.M.R. due to thyroidectomy; 3-6 wks.
4 c.c.Ext.A boiled 1 hr., subcutaneously	22	M	+ 34		None	
"	35	M	+ 29		8	
"	47	M	+ 11		5	
"	45	F	+ 17		24	
"	36	F	+ 8	+ 12	None	- 44
"	38	F	+ 9	+ 25	None	- 32
"	39	F	+ 18	+ 12	15	- 28
"	31	F	+ 21	+ 55	18	- 33
4 c.c.A.I.F. subcutaneously	43*	M	+ 26		None	
			Aver...+ 19	Aver..+ 26		

* R 43 received injections before operation. The day before showed + 28%, the day after + 25% and 2 days after +26%.

been injected for more than one month before thyroidectomy: removal of the thyroid did not alter the response on the first or second day after operation. Table 5 also shows a similar effect in some of the same animals after an interval sufficient to produce some thyroid insufficiency, as indicated by the B.M.R. The response in the latter group differed since the peak occurred nearer the sixth than the third hour, as in normal or recently thyroidectomized animals. It should be noted that the rise is relatively less than the actual figure + 26% would indicate, since it is calculated on a much lower pre-injection level. This appears to be fairly definite evidence that the pituitary gland contains a metabolic stimulant other than the thyrotropic hormone. The immediate metabolic stimulant does not produce the clinical signs of hyperthyroidism such as nervousness and restlessness, making weighing difficult. On the contrary, the animal remains quiet, with muscles relaxed. The increased depth and rate of respiration and an increased blood supply to the ears are the only evidence of increased metabolism. Furthermore, there is no clinical appearance of hypersecretion of adrenalin in which increased movements are fairly characteristic. It appears likely that the sudden increase in metabolism of rabbits (also of guinea

pigs and rats) described by Krogh and Okkels (1933) is due to the second factor, since the rabbits included in Table 4 were injected daily after the first injection, and study of the B.M.R. before injection showed that the rise in metabolism typical of the thyrotropic hormone does not appear until about the third day. Fig. I is a typical example of both metabolic hormones, in which the daily fluctuations of metabolism are represented by the vertical lines. An imaginary line joining the lower ends of these lines represents the typical thyrotropic effect. It is obvious that resistance was developed to the thyrotropic factor, but under the conditions of the experiment no resistance was developed to the factor producing the immediate rise.

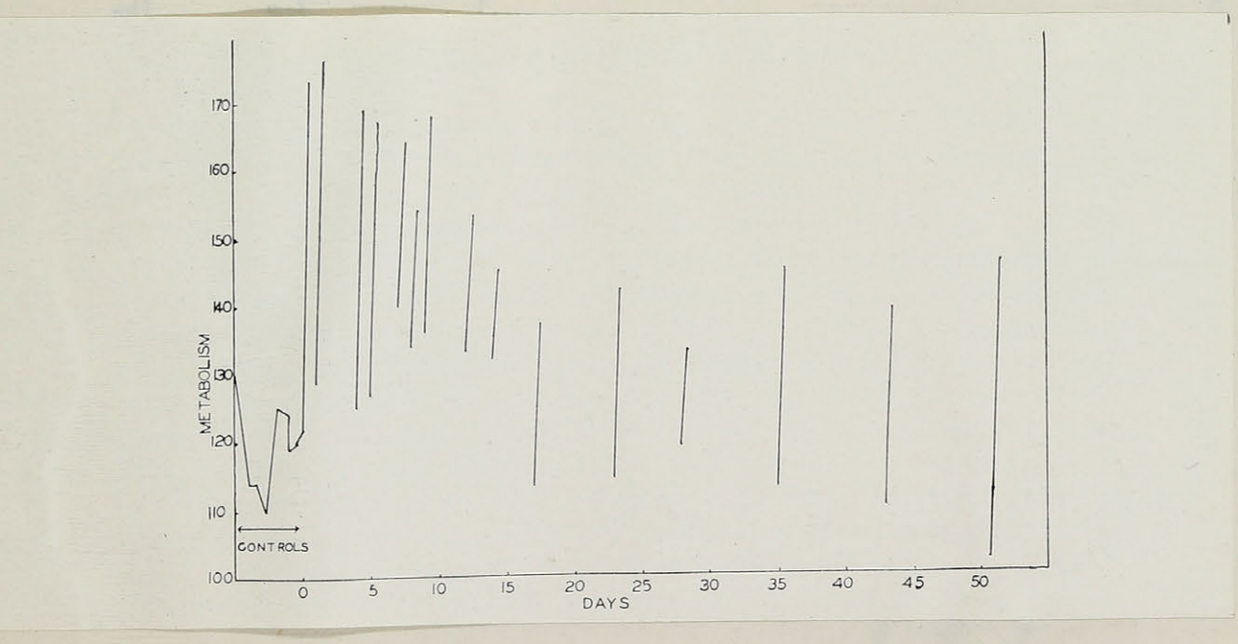


Fig. I. Influence on basal metabolism of 4 c.c. of anterior pituitary extract daily intraperitoneally. Metabolism is expressed as litres of oxygen per square metre of body surface per 24 hours.

TABLE 6.

INFLUENCE OF HEAT ON METABOLIC STIMULATION.

	4 c.c. Pituitary Extract A.	Rabbit Serial No.	Sex	Pre-injection level *	Post-injection Rise %
	Boiled 2½ hrs.	23	F	103	+32
	" " "	24	M	134	+19
	" " "	25	M	128	+19
	Boiled 1 hr.	26	F	125	+24
	" " "	26	F	128	+22
**	" " "	19	M	127	+25
**	" " "	16	F	114	+39
**	" " "	21	F	107	+49
				Average...	+28.7%

* Litres of oxygen/squ.metre body surface/24 hrs.

** R.19:16:21 were 3 chronically treated animals and controls with unboiled pituitary extract were respectively +47%: +42%: +44%.

TABLE 7.

EFFECT OF A SINGLE DAILY INJECTION OF 4 C.C. THYROTROPIC EXTRACT "A" (BOILED FOR 1 HR.) ON OXYGEN CONSUMPTION.

Rabbit # 29, Female.

No.of days injected:	1	2	3	4	10	17	24	31	38	45	49	63	72
Pre-injection level:	122	140	140	137	136	125	120	129	131	130	117	112	106
Post-injection max.:	169	173	178	159	158	157	168	174	157	156	165	159	153
Percentage change :	+39	+24	+27	+16	+16	+26	+40	+35	+20	+20	+41	+42	+44

Average of 5 controls (before treatment) = 131 (Max. 137; Min.122)

Rabbit # 28, Female.

No.of days injected:	1	2	3	4	9	16	23	30	37	42	50
Pre-injection level:	131	136	142	141	141	129	133	119	122	118	116
Post-injection max.:	173	164	190	133	176	163	163	186	169	154	153
Percentage change :	+32	+21	+34	- 6	+25	+26	+23	+56	+38	+30	+32

Average of 5 controls (before treatment) = 137 (Max.144; Min.133)

TABLE 8.

EFFECT OF FREQUENT INJECTIONS (THREE PER DAY) OF PITUITARY EXTRACT "A" ON OXYGEN CONSUMPTION TAKEN BEFORE AND THREE HOURS AFTER INJECTION.

Rabbit No.19. Male. Chronically injected for 2 months with a single daily injection. The normal oxygen consumption before treatment = 144 (average of 5). Max.155; Min.134.

Date:	25/10/-	26/10/-	27/10/-	28/10/-	29/10/-
1st inj.	118-172	116-166	138-173	117-145	131-143
2nd inj.	No inj.	.....	137-164	125-140	128-142
3rd inj.	No inj.	.....	.....	.....	.....

Heat Resistance of the Immediate Metabolic Stimulant.

Further evidence that the immediate rise is due to a factor other than the thyrotropic hormone is obtained from a study of the resistance of the active principle to high temperature. The thyrotropic hormone is destroyed in a short time by the temperature of the boiling-water bath (Anderson and Collip, 1934; Junkmann and Schoeller, 1932). Recent work from this department (Collip, 1937) requires the above statement to be qualified by stating the pH of the extract. This pituitary extract A, when boiled for one hour at approximately pH 7, contains no appreciable amount of thyrotropic hormone, as indicated by its failure to produce hyperplasia in the thyroid of treated guinea pigs. Table 6 shows that it is still active, in so far as the immediate rise in metabolism is concerned, even after 2½ hours' boiling.

The Effect of Chronic Injections: the Extinction Phenomenon with the Immediate Metabolic Stimulant.

Animals injected daily for about 10 days may show a diminishing response compared with the first day (see Fig. I and Table 7). This "extinction phenomenon" has unfortunately not been investigated fully. But the following observation on one rabbit is of interest (see Table 8). This animal had been chronically injected for two months with a single daily injection. When the number

of daily injections was increased to three, the immediate increase in oxygen consumption diminished very rapidly to an insignificant degree. A similar rapid onset of a refractory state occurred in a single animal, which had received a daily injection for three weeks, when the animal was fasted four days, with the usual daily injection. These isolated cases of resistance are worthy of more complete investigation which the author hopes to undertake in the near future.

It will be observed in Fig. I that after two weeks of daily injection, the pre-injection level of oxygen consumption fell to a level lower than the original controls. This might possibly be due to the formation of the anti-thyrotropic substance in the serum. To test this hypothesis, two rabbits were injected daily with extract A, boiled for 1 hour at pH 7. Table 7 indicates that they likewise showed a similar fall in the pre-injection level. They also showed evidence of the initial extinction phenomenon.

Anatomical Distribution of the Active Principle in the Pituitary.

The high concentration of melanophore-dilating hormone in the thyrotropic fractions suggested the possibility that the thermostable metabolic fraction might be identical with this hormone of the pars intermedia. Doctor Stehle, of the Department of Pharmacology, kindly placed some his melanophore preparation at our disposal. It was prepared from standard posterior pituitary powder (Stehle, 1936), by acetic acid

TABLE 9.

INFLUENCE OF STEHLE'S PURIFIED MELANOPHORE HORMONE ON METABOLISM.

Dose	Sex	Serial No.	Pre-injection Level	% Rise B.M.R.
2 mg.	M	19	130	40
"	F	21	128	16
"	F	16	133	14
"	F	21	114	18
"	F	28	126	20
"	F	29	124	28
0.5 mg.	M	42	115	15
2.5 mg.	F	52	92	61
				Average....26.5

TABLE 10.

DETAILS OF PITUITARY EXTRACTS CONTAINING THE SPECIFIC METABOLIC HORMONE.

Extracts and Source	Thyrotropic-A from ant.lobe of ox	A.I.F.from whole gland of sheep	Stehle's melanophore from post. pituitary powder	Pituitary colloid from ox	Extract of pars intermedia of ox	Poly-B pig anterior lobe
Method of Extraction	70% alcohol precipitate	90% alcohol precipitate	Acetic acid extract.Filtrate from absolute alcohol precipitate	Acetic acid extract	Acetic acid extract	Aqueous extract at pH 5
Minimal Frog Melanophore Unit	0.5 c.c.of 1/2500 = 3.6 γ	0.5 c.c. of 1/5000 = 0.67 γ	1 γ	0.5 c.c. of 1/50,000	0.5 c.c. of 1/20,000	0.5 c.c.of 1/15,000= 0.17 γ
Dose Producing Metabolic Increase	4 c.c.= 72 mg. 2 c.c. active	4 c.c.=25 mg. (prolonged effect) 1 c.c.active	2 mg.	1 c.c. 0.4 c.c.active	1 c.c.	1 c.c.= 5mg. prolonged effect
Equivalent	2 c.c.= 3 gm. anterior lobe	1 c.c.= 4 gm. whole pituitary	2 mg. = 500 mg. post.pituitary powder	0.4 c.c. = 9 mg. colloid	.....	1 c.c.=0.41gm.

TABLE 11.

EXTRACTS OF PARS INTERMEDIA AND PITUITARY COLLOID (OX).

Serial No.	Sex	Extract	Dose c.c.	Hours after injection	% Change B.M.R.	R.Q. Before	R.Q. After
42	M	Colloid (4)	0.5	2 - 4	+ 18	-	-
48	M	"	0.4	2 - 4	+ 23	-	-
49	M	"	0.4	2 - 4	+ 14	-	-
51	F	"	1.0	2 - 4 6 - 8	+ 75 + 41	.86	.76 .74
"	"	Colloid (32)	1.0	2 - 4	+ 4	.90	.90
"	"	"	1.0	2 - 4	+ 12	.79	.76
"	"	Pars Intermedia	1.0	2 - 4 6 - 8	+ 21 + 2	.91	.85 .85
"	"	Colloid (4)	1.0	2 - 4 6 - 8	+ 19 - 9	.89	.81 .90
53	F	Pars Intermedia	1.2	2 - 4 6 - 8	+ 30 + 14	.84	.77 .79
54	M	Pars Intermedia	0.5	2 - 4	+ 3	.75	.74

extraction, and precipitation by absolute alcohol. The filtrate was taken to dryness in vacuo, and shaken with methanol. The soluble fraction was added to four times its volume of ethyl acetate. The precipitate was extracted with methanol repeatedly. The insoluble residue contained one frog unit per γ, and was contaminated with traces of oxytocin and vasopressin. This preparation produced a definite increase in oxygen consumption of rabbits three hours after injection, in doses of 2 mg. Even 0.5 mg. caused a significant rise (see Table 9).

Dilute acetic acid extracts of ox pituitary colloid and dissected pars intermedia were obtained from Doctor Astwood (Johns Hopkins Hospital, Baltimore). They had already been assayed for their erythrophore activity (Lewis, Lee and Astwood, 1937). Their melanophore activity as assayed by us ran somewhat parallel with the erythrophore activity (see Table 10). Doses of 0.4 c.c. of colloid extracts, equivalent to 9 mg. of original colloid, stimulated oxygen consumption at the third hour. Extracts of the pars intermedia had a similar potency (see Table 11). This experiment is of considerable interest since the pars intermedia is the recognized source of the melanophore hormone, and the colloid is, from histological investigation, an accumulation of its secretion (Lewis and Lee, 1927; Cushing, 1933).

A single injection of an extract made from 200 mg. of anterior pituitary powder of the fin-back whale by boiling in N/10 NaOH for 10 minutes produced a significant increase in

oxygen consumption (+19%). Valsö (1934) and Geiling(1935) have shown that the anterior lobe of this species contains the melanophore hormone, whilst the anatomically separate posterior lobe contains both oxytocin and vasopressin.

Table 10 gives a summary of the various pituitary extracts, their source, method of extraction, melanophore hormone content, and effective metabolic dose. Owing to wide individual variation in animal sensitivity, a constant ratio cannot be expected. Nevertheless, the results indicate a relationship between the two substances.

Included in Table 10 are details of extracts A.I.F. and Poly-B (pig), showing the high melanophore content of both and their metabolic activity. Further details about these extracts will be given later (see below).

Effect of Posterior Lobe Hormones Oxytocin and Vasopressin on Oxygen Consumption.

Using Stehle's (1933) preparations known as Postlobin-O and Postlobin-V in doses of 15 to 30 units, the oxygen consumption of three rabbits was studied with each hormone (see Table 12). There was a well-marked fall in oxygen consumption in two cases with the latter, followed by a compensatory rise in one case. This type of reaction has already been described by Geiling and de Lawder (1932) in dogs, and Grollman and Geiling (1932) in humans, using a preparation obtained from Parke Davis and Company. Oxytocin (Postlobin-O) produces no significant alteration. The

TABLE 12.

INFLUENCE OF POSTERIOR LOBE HORMONES POSTLOBIN-O (OXYTOCIN) AND POSTLOBIN-V (VASOPRESSIN) ON OXYGEN CONSUMPTION.

Serial No.	Sex	Extract	Dose in units	Before Injection	1st hour	2nd hour	3rd hour	4th hour
28	F	Postlobin-O	15	124	126	126	123	---
29	F	"	15	127	125	122	121	121
16	F	"	30	110	107	119	112	116
28	F	Postlobin-V	15	123	131	127	124	131
29	F	"	15	120	101	---	122	---
21	F	"	30	110	100	113	107	---

FIGURE II.

EXAMPLES OF METABOLIC CHANGES WITH VARIOUS PITUITARY EXTRACTS.

(1) Rabbit No.9. Crude alkaline extract of sheep anterior pituitary; 5 c.c. intraperitoneally = 1 gm. of gland.

(2) Rabbit No.30. Crude alkaline extract of ox anterior pituitary; 5 c.c. intraperitoneally = 1 gm. of gland.

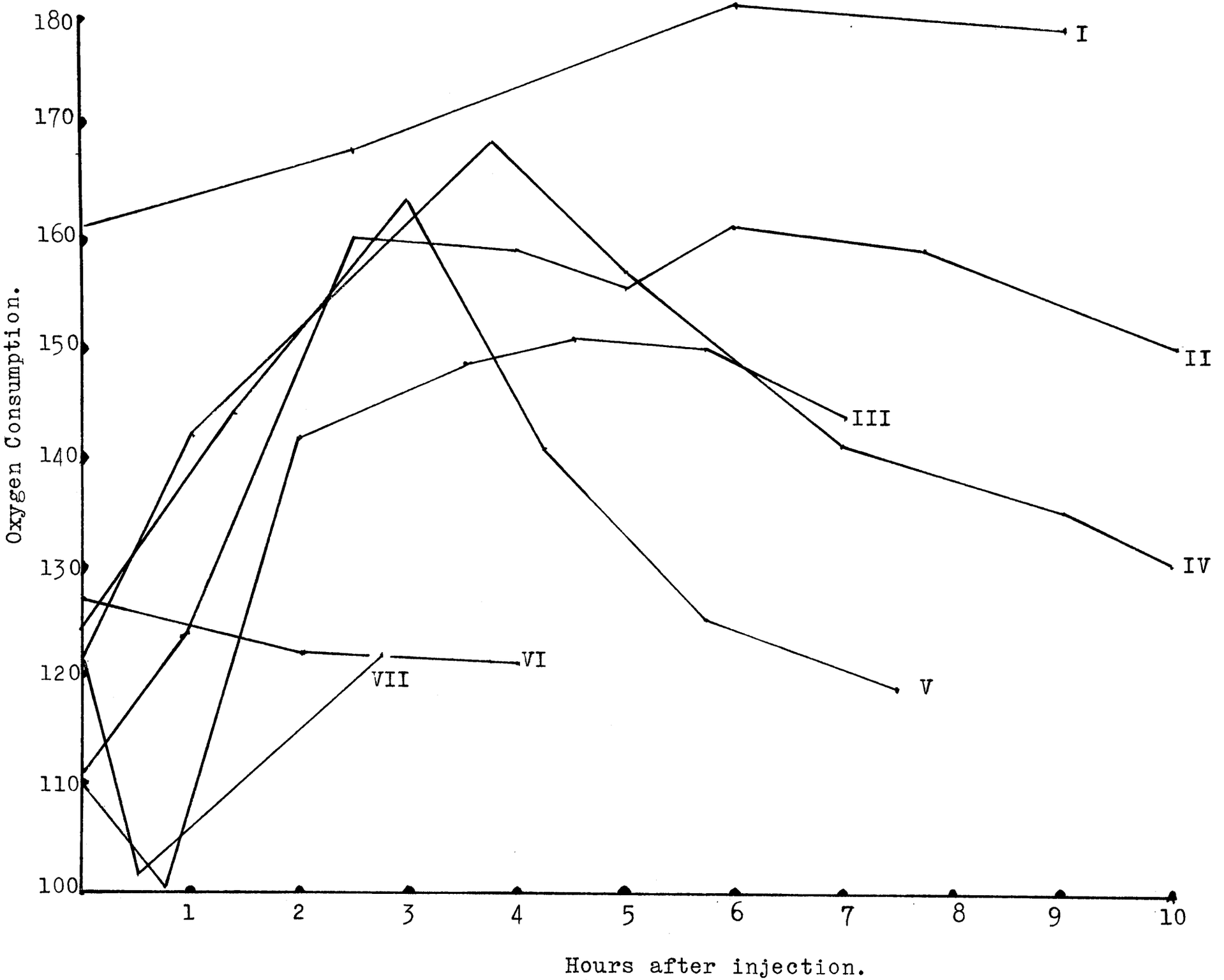
(3) Rabbit No. 16. 4 c.c. A.I.F. subcutaneously.

(4) Rabbit No. 29. Extract A; thyreotropic fraction, boiled at pH 7 for 1 hour; 4 c.c. subcutaneously.

(5) Rabbit No.29. 2 mg. Dr. Stehle's posterior pituitary melanophore powder subcutaneously.

(6) Rabbit No.29. Postlobin-O (oxytocin); 15 units subcutaneously.

(7) Rabbit No.29. Postlobin-V (vasopressin); 15 units subcutaneously.


180
170
160
150
140
130
120
110
100
Oxygen Consumption.
1
2
3
4
5
6
7
8
9
10
Hours after injection.
I
II
III
IV
V
VI
VII

immediate rise in metabolism already described with anterior lobe extracts is not preceded by a fall as occurs with Postlobin-V. Fig. II shows examples of the influence of various pituitary extracts on metabolism.

Some Physical and Chemical Properties of the Thermostable Metabolic Stimulant.

Since it is well established that the melanophore hormone is relatively resistant to alkali and may even be potentiated by boiling in N/10 NaOH for 15 minutes (Hogben and Gordon, 1930; Jores, 1933a; and Stehle, 1936), it was of considerable interest to investigate the reaction of the metabolic stimulant to this treatment. Table 13 includes data with various extracts boiled at pH 10 for 10 to 15 minutes. There is no evidence of a quantitative change. Two reasons may be advanced for these negative results. First, the extracts used had already been subjected to dilute alkali in the initial stage of preparation; and secondly, dosage used in controls was sufficient to raise the oxygen consumption to the "ceiling level" of metabolism. To avoid such objections, further experiments were conducted with an acid extract of anterior pituitary which at no time had been exposed to alkaline treatment. The dose given was submaximal. The data given in Table 14 appear at first sight to prove the hypothesis of alkali potentiation, but the author is not completely convinced that one is justified

TABLE 13.

INFLUENCE OF BOILING AT pH 10 FOR 10 MINUTES ON THE IMMEDIATE RISE IN CHRONICALLY INJECTED RABBITS.

Pit.Ext.	Dose c.c.	Sex	Serial No.	% Rise B.M.R. (unboiled)	% Rise B.M.R. (boiled pH 10)
M	4	M	42	31	30
M	4	M	49	20	23
A.I.F.	4	M	42	40	46
A.I.F.	4	M	49	27	27
Stehle's Melanophore Powder	3 mg.	F	52	Boiled in N/10 NaOH for 15 min.	41

TABLE 14.

EFFECT OF BOILING AN ACID EXTRACT AT pH 11 FOR 15 MINUTES.

Dose - 10 c.c. subcutaneously.

Serial No.	Sex		Pre-injection O_2 consumption.	% Change in O_2 consumption.
51	F	Control:	116	+12
		alk.treated:	115	+17
52	F	control :	101	+11
		alk.treated:	88	+35
		alk.treated:	109	+15
53	F	control:	110	+13
		alk.treated:	123	+15
		control:	112	+13
54	M	control:	116	+ 8
		alk.treated	102	+29

Average of controls =+11.4%
Average of alk. treated = + 22.4 %

TABLE 15.

INFLUENCE OF CHARCOAL ADSORPTION ON EXTRACT A.I.F.

Frog Unit Less than 0.5 c.c. of 1/100 Dilution.

	Sex	Dose	Hours after Injection	% Change B.M.R.	R.Q. Before	R.Q. After
R.52	F.	1 c.c. Filtrate	2 - 4	+ 2	.75	.78
"	"	3 c.c. "	2 - 4	+ 8	.91	.88
			6 - 8	+ 7		.81
"	"	* Control	2 - 4	+34	.80	.92
		3 c.c. A.I.F.	6 - 8	+39		.73

* Marked hyperpnoea during the first 3 hours after injection.

in drawing such a conclusion, because the pre-injection level varied in this group of animals in a rather bewildering fashion. It is remarkable that the alkali boiled extract showed no quantitative or qualitative change in the frog's melanophore.

The metabolic principle is easily soluble in water and insoluble in acetone. It usually retains its activity on storage at low temperature in 10 % alcohol even for as long as two years. But the potency of some extracts rapidly diminished during storage, for no apparent reason. Thus an extract Poly-B (May, 1937), after eight months' storage, had lost both its activity on the frog's melanophore and on metabolism (see Table 18). The metabolic principle is stable in dilute acids (pH 5); Astwood preparations of colloid were stored at pH 5 for six months without apparent loss of potency. It is adsorbed by charcoal, if the charcoal is slowly filtered off (see Table 15). However, if it is centrifuged off after 2 minutes at room temperature, the supernatant may contain an appreciable amount of melanophore and metabolic activity. Jores (1933 a or b) and Dietel (1933 a) have published different views on the adsorption of the melanophore hormone. The former held it to be an essential characteristic, while the latter claimed that it was adsorbed with difficulty.

TABLE 16.

EFFECT OF TRYPTIC DIGESTION AT pH 8.5 ON EXTRACT A.I.F.

Frog Unit Less than 0.5 c.c. of 1/100 Dilution.

	Sex	Dose	Hours after Injection	% Change B.M.R.	R.Q. Before	R.Q. After
R.51	F	1 c.c.	2 - 4	+36	.87	.84
"	"	1 c.c.+ Trypsin	2 - 4	+ 1	.95	1.02
			6 - 8	+ 5		.91
R.54	"	1 c.c.	2 - 4	+37	.72?	.78
			6 - 8	+ 4		.76
"	"	1 c.c.+ Trypsin	2 - 4	+ 1	.85	.88
			6 - 8	+ 1		.83
"	"	4 c.c.+ Trypsin	2 - 4	- 3	.93	1.01

Both activities are abolished by prolonged tryptic digestion - 48 hours at pH 8.5 (see Table 16) - but in some earlier experiments, after 12 hours' digestion there was only a negligible loss (see Table 22 , where such data are indicated by partial digestion). These findings are not in agreement with Hogben and Winton (1922) who found a rapid destruction of the melanophore-dilating hormone. Possibly the low efficiency of the trypsin used in our experiments, or an inhibiting effect of the $(NH_4)_2SO_4$ present in the extract, may have influenced our results. This explanation is suggested by the slow digestion of the Metz tube. Both principles are resistant to pepsin at pH 3 for 1½ hours (see Table 22).

The Thermostable Metabolic Principle and Carbohydrate Metabolism.

The changes in blood sugar concentration during the increased metabolism were studied as an indication of the possible secretion of adrenalin. Eight rabbits of known sensitivity were injected with various preparations of the active principle, and the data summarized in Table 17 show a small but well-defined fall in blood sugar concentration coinciding with the increased oxygen consumption. Control subcutaneous injections of adrenalin in amounts insufficient to increase the B.M.R. cause a marked hyperglycaemia. Zunz and Labarre (1934) and Horster (1933)

TABLE 17.

EFFECT OF PITUITARY EXTRACTS ON BLOOD SUGAR.

Average of 8 experiments on 8 rabbits.

Extracts used: 4 c.c. anterior pituitary A (2); boiled at pH10 for 15 min.
4 c.c. anterior pituitary M (4); " " " " " "
3 mg. Stehle's posterior pituitary melanophore (2); boiled in N/10 NaOH for 15 min.

Before Injection	First hour	Second hour	Third hour	Fourth hour	Fifth hour
Av.129 (Max.143 / Min.117)	Av.116 (Max.127 / Min.101)	Av.111 (Max.120 / Min. 84)	Av.107 (Max.120 / Min. 98)	Av.115 (Max.122 / Min.105)	Av.122 (Max.131 / Min.113)

TABLE 18.

INFLUENCE OF PITUITARY METABOLIC PRINCIPLE ON LIVER GLYCOGEN.

Sex	Fasted (hrs.)	Extract (Intravenously)	Before Inj. %	2 hrs. after Inj. %	Blood Sugar Conc. Before	Blood Sugar Conc. 2 hrs. after
F	14	3 mg. Stehle's Melanophore extract	5.904	5.658	.141	.140
F	14	2 mg. "	3.090	3.270	.128	.100
F	0	2 c.c. Extract No. I*	16.700	15.100	.132	.130

Average % change in liver glycogen after 2 hrs. = - 2.7

* Dry acid alcohol extract by Dr. O.F. Denstedt.

have attributed a hypoglycaemic action to the thyrotropic hormone. It is possible that their results might be due to contamination with the immediate metabolic factor.

For the study of blood sugars, extracts made from the dissected anterior lobe were designedly used, owing to the variable influence of posterior pituitary extracts on blood sugar. The posterior lobe melanophore preparation caused a similar drop in blood sugar after boiling in N/10 NaOH for 15 minutes (see Table 17).

The changes in liver glycogens following intravenous injection of the active principle into rabbits under amytal anaesthesia were studied on three rabbits. The change after two hours is almost within the limits of experimental error(Table 18) Further study of the other phases of carbohydrate metabolism was not pursued since Doctor Neufeld of this department undertook a more detailed study of the problem. His results (in press) on liver and muscle glycogen and blood sugar concentration are in agreement with the above findings that carbohydrate metabolism is depressed. In at least one case he found evidence which suggested that carbohydrate stores were actually increased three hours after intravenous injection of a potent extract(?gluconeogenesis).

Various authors have described an accumulation of carbohydrate stores following injection of pituitary extracts: the "glycostatic" effect of Russell and Bennett(1936); the "glycotropic" effect of Young (1936); the carbohydrate inhibitory effect of Meyer, Wade and Cori (1937) and of Russell (1938).

TABLE 19.

24 HOUR URINARY NITROGEN EXCRETION OF RATS WITH 4 INJECTIONS OF 0.5 c.c.A.I.F. (BOILED AT pH 10 FOR 10 MINUTES AND pH 7 FOR 1 HOUR). ANIMALS FASTED 48 HOURS BEFORE INJECTION. NITROGEN EXPRESSED IN GMS.

Second day	Injected during third day	Third day	% Change
Average of 5 = 145.2	2 c.c. A.I.F.	141.2	-2.6
Average of 6 = 111.7	2 c.c. 0.5 % $(NH_4)_2SO_4$	115.2	+2.7

TABLE 20.

CHANGES IN BLOOD UREA CONCENTRATION AFTER INTRAVENOUS INJECTION OF 1 c.c. POLY B EXTRACT (PIG).

Boiled at pH 7 for 1 hr. and pH 10 for 15 min. Rabbits fasted 12 hrs.

Serial No.	Sex	Blood urea conc. before injection mg./100 c.c.	Blood urea conc. at 5th hr. mg./100 c.c.
51	F	41.94	37.45
56	F	42.54	35.95
57	F	39.55	35.95
52 *	F	41.95	41.20
52a*	F	40.85	40.35

* Two rabbits whose oxygen consumption was not significantly increased after injection. One had middle-ear disease (52 a).

TABLE 21.

CHANGES IN BLOOD UREA OF TWO DOGS FASTED 48 HOURS BEFORE INJECTION.

Serial No.	Weight kilos.	Extract	Dose c.c. subcut.	Blood urea conc. before injection mg./100 c.c.	Blood urea conc. at 7th hr. mg./100 c.c.
8	24.2	Poly B (ox) boiled at pH 7 and 11	25	18.5	13.2
7	22.0	No. I D	22	31.76	23.67
7	22.0	Poly B (pig) boiled at pH 11 for 15 min.	24	21.00	16.00

The Thermostable Metabolic Principle and Nitrogen Metabolism.

Owing to the difficulty of obtaining accurate urine samples from rabbits, the effect of extract A.I.F. on nitrogen metabolism was studied on other species. Two groups of rats, 6 in each, were fasted 4 days and during the 4th day one group received 4 injections of 0.5 c.c. of A.I.F., whilst the control group received 0.5 .c.c of 0.5 % ammonium sulphate. The results are summarized in Table 19. As an index of gluconeogenesis from protein, the blood urea concentrations of rabbits and dogs were studied (Tables 20 and 21). There is a definite decrease in both cases, whilst two rabbits which were resistant to metabolic stimulation did not show a corresponding fall in blood urea. In general the evidence suggests that protein catabolism is depressed. A similar effect of pituitary extracts has been described by other workers who usually attributed it to stimulation of growth (Gaebler, 1933 and 1935; Gaebler and Price, 1937; Shaffer and Lee, 1935; Reiss, Schwartz and Fleichmann, 1936; and Mirsky and Swadesh, 1938).

The Influence of the Thermostable Metabolic Stimulant on Respiratory Quotient and Comparison with Adrenalin.

Unfortunately the R.Q. was not studied in much of the earlier experimental work detailed above, because the author considered the metabolic effect too transient in character to give reliable results. However, Doctor Collip

TABLE 22.

EFFECT OF EXTRACT A.I.F. ON GASEOUS METABOLISM AT THE 6TH TO 8TH HOUR AFTER INJECTION.

Serial No.	Sex	Dose c.c.	Treatment of Extract	Hours Fasted	Pre-injection level of O_2 consumption	% change in O_2 consumption 6th-8th hr.	R.Q. Before	R.Q. After
50	M	4	Boiled at pH7(1hr.)pH11(15min.)	2	139	+ 3	1.06	1.01
"	"	"	" " " " " "	23	113	- 3	.92	.92
"	"	"	" " " " " "	36	128	+48	.78	.74
51	F	"	" " " " " "	2	129	+36	.99	.79
"	"	"	" " " " " "	2	155	+25	.95	.80
"	"	"	" " " " " "	28	134	+27	.80	.73
"	"	"	Partial tryptic digestion	32	106	+ 4	.83	.83
"	"	"	48 hrs. tryptic digestion	16	116	+ 5	.95	.91
52	"	"	Partial Pepsin digestion	14	109	+22	.84	.80
"	"	"	Partial tryptic digestion	38	110	+ 6	.77	.77
"	"	"	Partial charcoal adsorption	12		+38	.83	.76
"	"	3	.....	42	103	+39	.80	.73
53	"	4	Partial tryptic digestion	14	111	+24	.81	.75
"	"	"	Partial peptic digestion pH2.0	38	114	+26	.74	.72
"	"	"	Partial tryptic digestion	18	105	+24	.91	.78
"	"	"	.....	15	112	+37	.87	.76
54	M	1	48 hrs. tryptic digestion pH8.5	16	119	+ 1	.85	.83
"	"	"	.....	40	115	+ 4	.72?	.76
"	"	4	Partial Norite adsorption	56	102	+33	.74	.73
"	"	"	Partial tryptic digestion	26	106	+ 9	.76	.75

FIGURE III.

The relation of oxygen consumption to R.Q. at the 6th to 8th hour after injection of 4 c.c. of Extract A.I.F. subcutaneously. The animals were fasted 2 - 48 hours. The data include two which were obtained with peptic digest of the extract (+ 22 and + 26 %).

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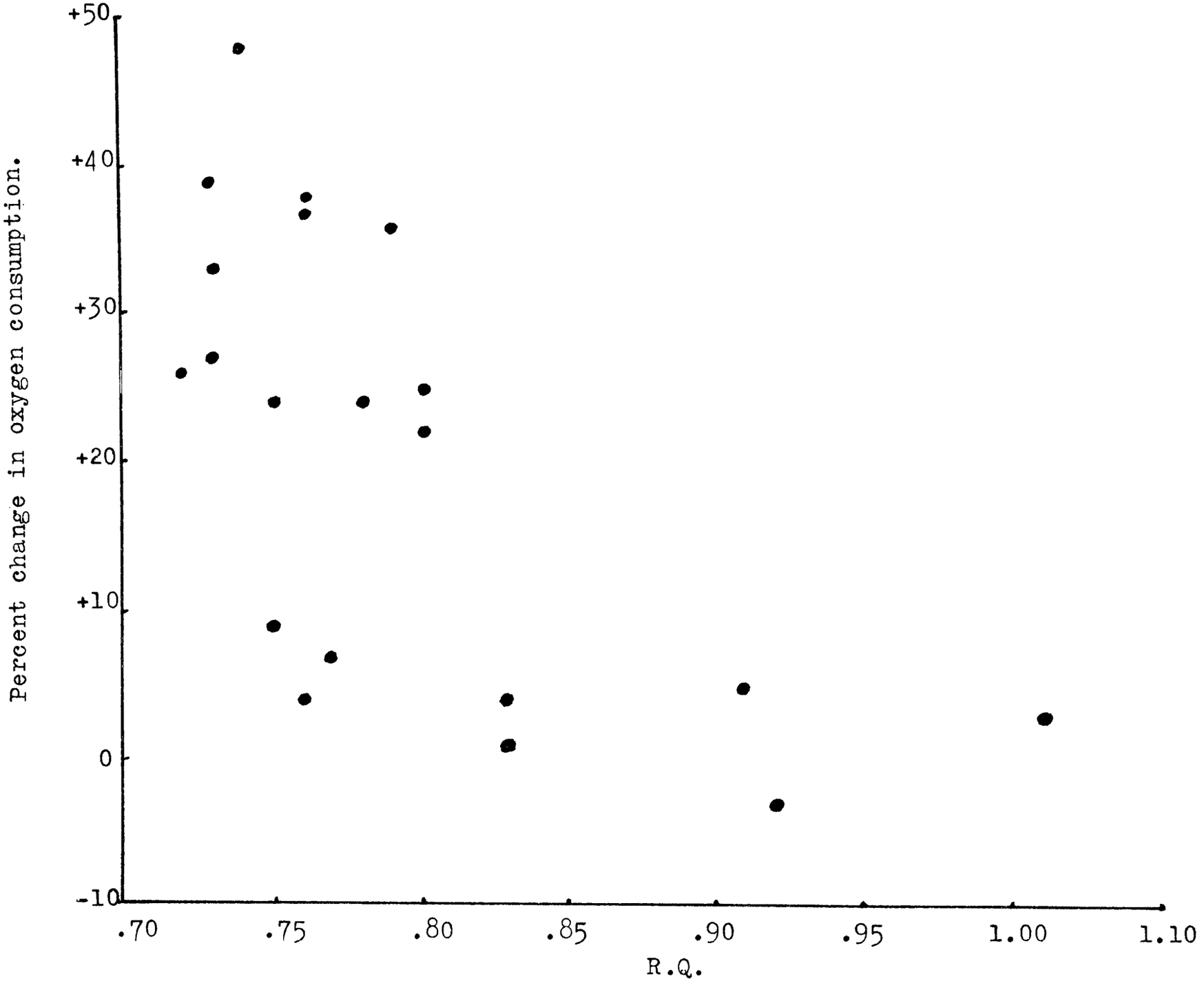
+50
+40
+30
+20
+10
0
-10
Percent change in oxygen consumption.
.70
.75
.80
.85
.90
.95
1.00
1.10
R.Q.

prepared two extracts termed "A.I.F." and "Poly-B" which gave a prolonged increase in metabolism (8 hours) with ordinary doses. This made possible a more complete investigation of the gaseous metabolism.

If one makes allowance for certain disturbing factors such as blowing off of CO_2 , the R.Q. shows a definite fall with the increased metabolism. Table 11 gives the R.Q. values with preparations of pars intermedia and pituitary colloid. Fig.III and Table 22 show clearly the relationship between the oxygen consumption and R.Q. at the 6th to 8th hour after injection of A.I.F. with varying periods of fasting (2 to 56 hours). The data obtained with this extract at the 2nd to the 4th hour after injection show a less evident correlation due to blowing-off of CO_2. This effect may be due to the inorganic constituents, since the tryptic digest and filtrate from charcoal sometimes produce a rise in R.Q. without a significant change in oxygen consumption (Tables 15 and 16). This extract lowers the CO_2 combining power of plasma by 11 c.c. p.c. (average of 8 determinations: max.14.6; min.6.6). The immediate effect on the R.Q. is more clearly demonstrated by the data obtained with Poly-B (an extract of pig pituitary)

TABLE 23.

EFFECT OF INTRAVENOUS INJECTION OF EXTRACT POLY B(PIG).

(Boiled at pH7 for 1 hr.& pH11 for 10 min.) Dose = 1 c.c.

Serial No.	Sex		Before Injection	Time in hours after injection ¼ - 2	2 - 4	4 - 6	6 - 8	22 - 24
51	F	O_2	109	137	144	138	128	-
		R.Q.	.89	.75	.74	.77	.76	-
51	F	O_2	120	130	139	127	-	119
		R.Q.	.96	.83	.80	.85	-	.81
56	F	O_2	133	154	167	-	-	-
		R.Q.	.92	.88	.78	-	-	-
56	F	O_2	127	152	-	153	121	119
		R.Q.	.98	.82	-	.83	.82	.82
57	F	O_2	103	136	137	131	115	109
		R.Q.	.94	.78	.77	.81	.84	.85
57	F	O_2	134	-	187	171	-	105
		R.Q.	1.04	-	.81	.87	-	.86
Average of fed group		O_2	121	142	155	144	121	113
		R.Q.	.955	.812	.780	.826	.807	.835
51	F	O_2	110	138	136	132	127	
		R.Q.	.78	.75	.75	.75	.75	
51	F	O_2	93	118	121	121	121	
		R.Q.	.81	.75	.77	.77	.77	
57	F	O_2	102	-	144	-	135	
		R.Q.	.78	-	.73	-	.72	
56	F	O_2	120	127	129	131	124	
		R.Q.	.79	.75	.77	.77	.76	
Average of fasted group		O_2	106	128	135	128	127	
		R.Q.	.790	.750	.755	.763	.750	
58 Hypophysec-tomized	F	O_2	118	120	116	116	111	
		R.Q.	.85	.77	.76	.75	.74	
58 "	F	O_2	108	112	111	117	-	
		R.Q.	1.00	.80	.83	.79	-	

TABLE 24.

THE INCREASED CO_2 EXCRETION DURING THE IMMEDIATE INCREASE IN OXYGEN CONSUMPTION AFTER VARYING PERIODS OF FASTING.

Serial No.	Sex	Hours fasted	Hours after injection	M.R. % Change	Increase in CO_2 Excretion Gm.
51	Female	2	2 - 4	+ 20	.238
			6 - 8	+ 36	.162
53	Female	14	2 - 4	+ 8	.331
			6 - 8	+ 24	.366
52	Male	24	2 - 4	+ 24	.673
			6 - 8	+ 22	.475
54	Male	56	2 - 4	+ 27	.864
			6 - 8	+ 33	.823

after intravenous injection (Table 23). This extract contains no ammonium sulphate and was prepared from dissected anterior lobes; it may be considered relatively free of posterior lobe hormones. It lowers the CO_2 combining power of plasma by 3.5 c.c. p.c. (average of 5). The results show the instantaneous drop in R.Q. which is especially evident in well-fed animals. The percentage increase in oxygen consumption is approximately the same in both fed and fasted animals.

Another method of expressing the fact that the R.Q. falls less in a fasted than in a fed animal is to compare the increased CO_2 excretion found after different periods of fasting. Table 24 gives examples of four animals after their <u>first</u> injection of a pituitary extract (A.I.F.). There is a remarkable increase in the excess CO_2 given off in the fasted compared to the non-fasted animals. This point is of particular interest since a fall in R.Q. with an increased oxygen consumption could theoretically be attributed to a transformation of fat to carbohydrate (Cathcart and Marcowitz, 1927). But if this form of gluconeogenesis were one hundred per cent efficient there should be no increase in CO_2 output.

It should be observed that animals not previously treated were chosen particularly to express the changes in CO_2 excretion, since chronically injected animals have shown a tendency to an abnormally high R.Q. after feeding. This

TABLE 25.

INFLUENCE OF ADRENALINE ON B.M.R. AND R.Q. AT VARYING INITIAL LEVELS OF R.Q.

Serial No.	Sex	Dose mg.	Time after injection (hours)	% Change B.M.R.	R.Q. Before	R.Q. After
51	F	0.5	2 - 4	+ 2	.99	.98
"	"	"	2 - 4	+16	.90	.89
			6 - 8	+ 5		.84
"	"	"	2 - 4	+33	.84	.91
"	"	1.0	2 - 4	+24	.73	.78
52	"	0.5	2 - 4	+32	1.04	.96
			6 - 8	+14		1.01
"	"	"	2 - 4	+20	.78	.89
53	"	"	2 - 4	+31	.93	.91
56	"	1.0	2 - 4	+21	.81	.81
57	"	0.5	2 - 4	+23	.86	.86
		Average of	2 - 4 hr. data	+22.4%	.876	.888

TABLE 26.

EFFECT OF VARIOUS PITUITARY EXTRACTS, WHICH ARE LOW IN MELANOPHORE ACTIVITY, ON GASEOUS METABOLISM.

Serial No.	Extract	Dose c.c.	Time after injection hrs.	% Change in O_2 consumption	R.Q. Before	R.Q. After
51	Growth Q 10	10	2 - 4	+ 1	.80	.81
55	1.5 N dry Ammoniacal alcohol	2	2 - 4	- 5	.78	.76
51	Extract 10	4	2 - 4	0	.75	.79
51	1.5 N dry Ammoniacal alcohol	5	2 - 4	- 3	.84	.83
42	Prolactin (of Mar.5th)	4	2 - 4	-29	-	-
21	Iso-electric precipitate = G.A.P.preparation.	4	2 - 4	- 9	-	-
21	Iso-electric filtrate (IA)=G.A.P.preparation.	4	2 - 4	+10	-	-
51	Poly-B (pig) (May,1937)	4	2 - 4	+ 4	.86	.84

compensatory elevation of the R.Q. will be more completely studied in the future. For our present purpose it should, however, be pointed out that normally there is no compensatory rise after injection if food is withheld (see Table 23 on the 6 to 8 hour and the 22 to 24 hour data). This fact suggests that an irreversible reaction has caused the lowering of the R.Q.

Adrenalin controls included in Table 25 give a very different response, as one would expect from the work of previous authors. This study was undertaken at different fasting levels to stand comparison with the results obtained with extract Poly-B. The only instance where there is an appreciable lowering of R.Q. is in the fed animal with an abnormally high R.Q. (1.04). Such high respiratory quotients are generally considered to be due to transformation of carbohydrate to fat (Krogh and Lindhard, 1920). The fall in R.Q. from 1.04 to .96 merely indicates a temporary diminution of this process. There is no evidence to support the hypothesis of Cori and Cori (1928) that adrenalin stimulates fat oxidation.

The gaseous metabolism was studied under similar conditions with a wide variety of pituitary extracts. Unfortunately, owing to the tendency of the melanophore hormone to be adsorbed on protein precipitate, most of such extracts contained an appreciable quantity of this hormone. Table 26 gives some data obtained with extracts which happened to contain a negligible quantity. The changes in R.Q.

TABLE 27.

RECTAL TEMPERATURE (F.) AFTER INTRAVENOUS INJECTION OF 1 C.C. POLY B EXTRACT (PIG).

RABBITS FASTED 12 - 24 HOURS.

Serial No.	Sex	Before Inj.	Hours after Injection								
			1	2	3	4	5	6	7	8	9
51	F	102.6	101.8	-	103.6	105.0	106.1	105.7	-	-	103.0
56	F	102.1	102.5	-	104.1	104.3	104.6	104.2	-	-	103.1
60	F	102.3	102.4	104.5	103.9	102.9	-	-	-	-	-
57	F	101.5	101.0	102.1	103.0	103.3	-	104.0	-	102.8	-
61	F	101.7	102.0	103.1	101.9	101.9	-	-	-	-	-
Average of 5 controls		102.6	102.7	102.7	102.7	-	102.6	102.6	-	-	-

are in no case very considerable. The single instance where prolactin caused a dramatic fall in oxygen consumption is of interest in view of Riddle's, et al (1936) claim that their preparation stimulated metabolism. However, this result is not incompatible with their findings, since they did not study the immediate effect on metabolism. The data also exclude the likelihood that the metabolic effect under consideration is caused by either growth-promoting, adrenotropic or lactogenic hormones since the extracts referred to as G.A.P. were potent preparations of these hormones.

Body Temperature Changes with the Immediate Metabolic Principle.

In view of the increase in oxygen consumption, it was of interest to study the changes in rectal temperature. Studies on five rabbits are given in Table 27 The variable degree of hyperthermia is of slow onset and outrules the possibility that a sudden change in the heat-regulating mechanism may be the cause of increased metabolism. The same five animals were used as controls to exclude the influence of diurnal variation on temperature.

The Thermostable Metabolic Factor Differentiated from the Adrenotropic Hormone.

The possibility that the melanophore-dilating hormone and the adrenotropic hormone are one has been discussed. The association between the two effects is based on the relation of the adrenal cortex to pigmentation, and the fact that injections of intermedin or melanophore preparations are known to produce hypertrophy of the adrenal cortex (Holmquist, 1934; Jores and Beck, 1934); the latter authors also point out the relationship between basophilic adenomas of the pituitary and hypertrophy of the adrenal cortex as of some significance since basophilic tumours may have a high concentration of melanophore hormone. The only publication devoted to a differentiation of these two hormones is by Jores (1935). He found that in contradistinction to the melanophore hormone, the adrenotropic activity is not lost by charcoal adsorption. He used the method of Anselmino and Hoffmann (1934), i.e.normal young white mice, for assay of adrenotropic activity. Since this method of assay is considered unreliable (Collip, 1935), the author considered it worthy of investigation, using the hypophysectomized rat as a more suitable test object.

TABLE 28.

DIFFERENTIATION OF METABOLIC PRINCIPLE FROM ADRENOTROPIC.

Extract A.I.F. boiled at pH 10 for 15 min. and pH 7 for 1 hr.

Dose: 0.5 c.c. b.i.d. for 5 days, to rats hypophysectomized 2 months previously.

Rat No.	Wt. of right adrenal before injection - (mg.)	Wt. of left adrenal on 6th day - (mg.)	Final Wt. of rat. (gm.)
1	7	5	154
2	4	6	158
3	-	3	161
4	3	5	149
5	5	5	142
6	3	4	150
7	4	4	136
Average=	4.3	4.6	

TABLE 29.

DIALYSIS OF MELANOPHORE HORMONE AND METABOLIC PRINCIPLE.

Extract	Method of Dialysis	Serial No.	Sex	Dose of Dialysate subcutaneously c.c.	R.Q. Before	R.Q. After	% Change in oxygen consumption	Melanophore Activity
Poly-B (pig) Jan./38.	Collodian membrane	51	F	4	.89	.78	+ 32	0.5 c.c. 1/20,000 in H_2O
622 (8)	Electro-dialysis* to Anode	51	F	7	.87	.86	+ 6	0.5 c.c. 1/100 in H_2O.
622 (8)	Electro-dialysis to Kathode	51	F	5	.86	.79	+ 47	0.1 c.c. 1/20,000 in H_2O

* The author is indebted to Dr. O.F.Denstedt for the samples obtained by electro-dialysis.

The extract A.I.F. which is active on both rabbits' metabolism, and on the frog's melanophore in high dilution, was boiled at pH 11 for 15 minutes and at pH 7 for 1 hour. This treatment did not influence either of the above activities. It was then assayed for adrenotropic action on hypophysectomized rats (see Table 28). The right adrenal was first removed and weighed; the rats were then injected for 5 days with the treated A.I.F. extract, and killed on the 6th day. The weights of the left adrenals were not appreciably changed. This is considered fairly convincing evidence that the adrenotropic hormone is a separate entity.

Dialysis of the Melanophore Hormone and Metabolic Stimulant.

The extract Poly-B (pig) was dialyzed through a collodion membrane (see Table 29), and the dialysate contained a high proportion of melanophore-expanding hormone and the metabolic stimulant. Electrodialysis was performed by Doctor O.F.Denstedt, using fish-membranes; the kathode dialysate contained both factors, while the anode dialysate gave almost negative results. This is rather surprising in view of the findings of previous workers on the dialyzability of the melanophore-expanding hormone.

Concurrent Work Done in this Department with the Thermostable Metabolic Stimulant of the Pituitary.

At the 1938 meeting of the American Physiological Society, Doctor A.H.Neufeld and Doctor J.B. Collip reported further work done with the concentrated melanophore preparations such as A.I.F., Poly-B, and Extract 622. They produced the following effects: hyperglycaemia and glycosuria in Houssay dogs; ketonemia in fasted rats; a contra-insulin effect on blood sugar and onset of convulsions in rabbits; and finally, the power to neutralize the hyperglycaemia of adrenalin in rabbits. The active principle in each case had the following properties in common with the melanophore hormone: it was destroyed by trypsin, adsorbed by charcoal, dialyzable, and withstood the temperature of the boiling-water bath at pH 7 for 1 hour and pH 11 for 15 minutes.

Doctor L.W.Billingsley, in his Ph.D. thesis (1937) reported on the immediate metabolic stimulation with thyrotropic preparations using guinea pigs and hypophysectomized rats. Normal rats gave a relatively slight response with such extracts.

DISCUSSION OF THE EXPERIMENTAL FINDINGS.

The separate identity of the thermostable metabolic stimulant is apparent from the evidence already given. The only well-established hormone not already differentiated is the gonadotropic hormone. This is destroyed rapidly by boiling at pH 10 or 11 for 10 minutes, and even boiling at pH 7 or 8 for 1 hour almost completely abolishes its activity (Collip,1937). Furthermore, the very potent preparation A.I.F. was obtained from the supernatant of the 70 % alcohol precipitate. This precipitate is already known to contain a high proportion of the gonadotropic and thyrotropic hormones. Finally, there is no evidence that extracts of sheep pituitaries are a richer source of the metabolic stimulant than those of ox or pig. In fact the ox preparations of thyrotropic hormone were very active on metabolism although they contained little gonadotropic activity.

The close similarity of the metabolic stimulant to the melanophore-dilating hormone is striking. They have a similar anatomical distribution in the various parts of the pituitary. Both withstand the action of alkali at the temperature of the boiling-water bath. They are each adsorbed by charcoal and digested slowly by trypsin, but not by pepsin. We have so far obtained no convincing evidence

that the metabolic effect is potentiated by alkali, as is reputed to be the case with the melanophore-dilating hormone; however, we are not convinced that this property is an invariable characteristic of all melanophore preparations. On the other hand, it should be observed that the methods of assay for both admit of wide variation, making accurate comparison very difficult. The evidence that each activity is dialyzable, and in the case of electrodialysis that both are found at the kathode and neither at the anode is particularly convincing.

In view of the different effects of adrenalin and the pituitary metabolic factor on R.Q. and on blood sugar, it is suggestive that adrenalin may neutralize the action of the melanophore hormone on melanophores or retinal pigment of frogs' eyes (Jores and Caesar,1935).

Assuming that there are two chromatophore hormones in the pituitary gland, one acting on frogs' melanophores and the other on erythrophores of certain fish, the latter may be differentiated from the metabolic factor by its susceptibility to alkali. Stehle's preparation of melanophore powder is active in doses of even 1 γ on both frogs and fish (phoxinus). However, after boiling in N/10 alkali for 15 minutes, the preparation loses 9/10ths of its erythrophore activity (Stehle, 1938). This treatment causes no diminution of its metabolic activity.

Some extracts, particularly A.I.F., appear to have a more prolonged metabolic action than their melanophore content would suggest. This may possibly be due to the associated protein causing slower absorption and utilization.

Since Doctor Neufeld, of this department, has shown that the hyperglycaemia of adrenalin may be neutralized by this pituitary fraction, it becomes necessary to reconsider the possibility that the metabolic stimulation is due to adrenalin secretion. The following evidence is against such an hypothesis. The respiratory quotients of adrenalin controls show no significant drop after injection. The pituitary fraction causes only slight blowing-off of CO_2. From the work of Doctor Neufeld, the muscle glycogen remains intact after injection of such pituitary extracts. Finally, there is no evidence available that there is any other hypertensive substance in pituitary extracts other than vasopressin. Stehle's melanophore preparation is almost devoid of such action, and one of our preparations from anterior lobe (622 pig) had no significant effect on the blood pressure of a human after injection of 1,000,000 frog units intramuscularly. In general, although the evidence is very much against adrenalin being the immediate cause of the effects described, more direct proof is desirable, and work is at present in progress with this point in view. Demedullation of the adrenals in rabbits and adrenalectomy in rats or guinea pigs should help to decide the question.

The question arises whether the fall in R.Q. with an increased oxygen consumption can be interpreted in the above experiments as an increase of fat oxidation. Another theoretical possibility which must be considered is gluconeogenesis from fat (Cathcart and Marcowitz, 1927). This is of particular interest since Soskin, et al (1935) have postulated a deficiency of this mechanism in hypophysectomized dogs to explain various experimental findings. It is unlikely that this would explain all the experimental results described with the melanophore preparations for the following reasons. The R.Q. tends to fall to .71 but never below it. The fall in R.Q. is most marked in well fed animals and relatively slight in fasted animals, so that it is difficult to conceive why gluconeogenesis should be most pronounced when an excess of carbohydrate is available. In one hypophysectomized rabbit the R.Q. fell from 1.00 to .80 without any significant change in oxygen consumption. Lastly, the CO_2 excretion is increased, particularly in the fasted animal. If transformation of fat to carbohydrate is taking place, the conversion mechanism must be particularly inefficient at low initial levels of R.Q. - i.e. in fasted animals.

Since carbohydrate metabolism and nitrogen catabolism are not apparently stimulated but actually depressed to some extent, one is forced to the conclusion that fat oxidation is stimulated. This hypothesis accounts for all the data

obtained. The greater fall in R.Q. of fed animals is explained by the simultaneous depression of carbohydrate metabolism. Even in the fasted group the R.Q. falls more than one would expect from an increased fat oxidation. For example, in six experiments on fasted animals, the R.Q. fell from .768 to .739 and the oxygen consumption increased + 32%. Such an increase due to fat oxidation should depress the R.Q. to .753, if one assumes that carbohydrate and protein metabolism remain unchanged.

However, the assumption of an inhibition of carbohydrate metabolism based on estimation of carbohydrate stores before and after injection is open to an alternative explanation: namely, gluconeogenesis presumably from fat. It is possible that the fall in R.Q., greater than was expected on the assumption of increased fat oxidation, may be due to changes in the equilibrium of fat $\rightleftharpoons$ carbohydrate. At high initial levels of R.Q. possibly carbohydrate $\longrightarrow$ fat is inhibited and even reversed, so that the R.Q. would fall to a greater extent in fed than in fasted animals.

Hence it appears that more than one factor is operating in altered gaseous metabolism: first, an increased oxidation, presumably of fat; and secondly, either a depression of carbohydrate metabolism or increased gluconeogenesis.

DISCUSSION ON NOMENCLATURE, AND RELATION TO OTHER PITUITARY HORMONES.

The thermostable metabolic stimulant may be described as "the specific metabolic hormone of the pituitary". The title has a wide scope and does not associate it with any specific phase of metabolism. It does not stress the property of metabolic stimulation, which in some cases may be absent while the lowering of the R.Q. is still well marked. It also leaves the site of formation an open question until further proof is advanced that the pars intermedia elaborates such a hormone.

In the present state of our knowledge one should avoid the use of the name "intermedin" except in the original sense as used by Zondek and Krohn (1932) that it is a hormone which dilates the erythrophores of the fish phoxinus and is presumably elaborated by the pars intermedia.

The specific metabolic hormone of the pituitary is apparently a separate entity from the fat-metabolism hormone of Anselmino and Hoffmann(1931), which has no effect on R.Q., depresses the B.M.R. and is easily destroyed by heat. On the other hand the active principle described by Fisher, Russell and Cori (1936), Meyer, Wade and Cori (1937),and Russell (1938) appears to resemble the specific metabolic hormone of the pituitary, since it produces an increase in oxygen consumption of normal rats, with a lowering of the R.Q. and suppression of carbohydrate oxidation. However, it was easily destroyed by heat in each case.

DISCUSSION OF THE POSSIBLE ROLE IN THE BODY OF THE SPECIFIC METABOLIC HORMONE.

If one is correct in assuming that the specific metabolic hormone of the pituitary promotes the oxidation of fat while inhibiting the oxidation of carbohydrate metabolism, it is of interest to speculate on many problems in pituitary physiology. Thus the higher R.Q. of fasted hypophysectomized rats than that of normal controls may be due to a relative inability to utilize the fat reserves. Also, the hypoglycaemia on fasting, with rapid depletion of the carbohydrate stores found in many species after hypophysectomy, may be an expression of a similar deficiency. The alleviation of diabetes by hypophysectomy may also be due to removal of the source of the hormone which is normally forcing the metabolism towards fat oxidation. Thus the removal of both the pituitary and the pancreas leaves the organism in a state of unbalance between fat and carbohydrate oxidation. This conception is not wholly in accord with the over-production theory of diabetes. The facts are in agreement with the classical theory of diabetes, or a combination of the classical and the over-production theories, since the specific metabolic hormone may, in addition to stimulating fat oxidation, either depress carbohydrate metabolism or stimulate gluconeogenesis.

Many of the well known effects of pituitary extracts on intact or operated animals may also come within the scope of a hormone producing such an effect. As already mentioned (Doctor Neufeld), preparations of the specific metabolic hormone produce ketosis in fasted rats, hyperglycaemia, glycosuria and ketonuria in Houssay dogs, neutralize the hypoglycaemic action of insulin and the hyperglycaemic action of adrenalin. The glycostatic or glycotropic action of pituitary extracts may also be another effect of the same hormone.

It is not possible to say whether the active principle described is synonymous with the diabetogenic hormone of Houssay. Its contra-insulin effect suggests their identity. However, the final proof depends on chronic injections in normal dogs, with the production of a persistent glycosuria and increased nitrogen excretion.

SUMMARY.

A pituitary fraction is identified which stimulates oxygen consumption of rabbits within three hours after subcutaneous or intraperitoneal injection, and immediately after intravenous injection.

The rise in oxygen consumption is transitory in character but may last from 8 to 24 hours. The effect, within the limits studied, is independent of the nutritive state of the animal (2 to 48 hours' fast).

The rise in oxygen consumption is associated with a marked fall in R.Q., particularly if the animal is well fed. In one hypophysectomized rabbit, the oxygen consumption was not altered significantly although the R.Q. fell from 1.00 to .80. Adrenalin controls produce no significant change in R.Q.

The active principle is present in sheep, ox and pig pituitary glands. The pars intermedia and pituitary colloid contain the highest concentration, being about 100 to 200 times as rich a source as the dissected anterior lobe.

The active principle is associated with the melanophore-dilating hormone in various extracts. It requires the equivalent of about 5,000 frog units to stimulate the B.M.R. of a 2 kilogran rabbit to more than +15%; 50,000 units cause a prolonged effect over a period of about 8 hours.

The active principle resembles the melanophore-dilating hormone in its digestion by trypsin, resistance to pepsin, adsorption by charcoal, dialyzability, and resistance to heat and alkali.

Extracts of liver, kidney, muscle, pancreas and pineal gland do not simulate the effect of such pituitary extracts on gaseous metabolism.

The active principle is differentiated from oxytocin, vasopressin, prolactin, growth-promoting, adrenotropic, gonadotropic and thyrotropic hormones.

There is no evidence of increased nitrogen catabolism, and liver glycogen stores are not affected. The blood sugar concentration shows a slight fall. Body temperature shows a slight rise.

The evidence is discussed and the conclusion is arrived at that such extracts, apparently identical with the melanophore-dilating hormone, stimulate fat oxidation and simultaneously depress carbohydrate metabolism. The importance of such an active principle in the pituitary gland is discussed.

The title chosen to designate the active fraction is "the specific metabolic principle of the pituitary gland".

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