balances and reagent shelves and materially speeding up the laboratory work. The book is well printed and bound in attractively colored cardboard covers with a spiral binding so that the pages lie flat, even though the book is folded back cover to cover.

R. L. SHRINER

SPECIAL ARTICLES

A STUDY OF HORMONAL FACTORS WHICH INFLUENCE THE PRODUCTION OF INSULIN[1]

THE present work had its origin in the attempt to devise a method for diagnosing diabetic "tendencies" prior to the time when positive diagnosis is obtained with the routine tests, as gathered from analyses of blood, urine and glucose tolerance curves. It seemed possible that stimulating carbohydrate metabolism in "normal" subjects and in those with diabetic "tendencies" might reveal differences in hormonal relationship which could, perhaps, be detected by urine analysis.

Some preliminary work was necessary before embarking upon the more ambitious part of our program; and the present report deals with several interesting observations.

In a study of carbohydrate metabolism, involving the activity of hormones, we had to consider, aside from insulin, the diabetogenic hormone (D.H.) and the insulinotropic substance (I. S.).[2]

Methods of extraction and methods of estimation are given by Best, Haist and Ridout and by Campbell and Keenan.[3] Methods of estimation are based upon the following facts: the injection of an extract containing D. H. will decrease the amount of insulin in the pancreas, whereas the injection of an extract containing I. S. will increase the insulin content.

For the assay of the diabetogenic and insulinotropic effects, the rat method of Best[3] was used, ten male albino rats of 200–300 g in weight being injected intraperitoneally for a period of 14 days. The insulin assay in the pancreas of rats was carried out according to the directions of Marks,[4] using 40 mice.

Based on the work of Campbell and Keenan,[3] a fractionation procedure for the anterior pituitary was developed. These authors describe the preparation of an active extract of D. H. We were hopeful that the anterior pituitary would also yield an active extract of I. S.; and we therefore prepared fractions from the residue obtained after complete extraction of D. H. by a 10 per cent. salt solution. Four fractions prepared and tested were the following: 1. An alkaline extract of the glandular tissue which had previously been extracted with a solution of NaCl (fraction 2); 2. A globulin-like material, soluble in salt solution and insoluble after dialysis; 3. A fraction recovered from the solution remaining in the dialyzing bag after dialysis; 4. The combined dialysates after elimination of NaCl. In each case the fractions were concentrated *in vacuo* and precipitated with alcohol-ether.

Using 10 rats per fraction, amounts were injected equivalent to 10 g of the original anterior pituitary gland. From the table it can be seen that fraction 3 exhibits a slight diabetogenic effect, and that fraction 1 shows insulinotropic activity. The other two fractions were found to be inactive.[5]

Another phase of the subject was suggested by the work of Marks and Young,[6] who reported that crude prolactin preparations exhibited marked insulinotropic effects, although they were of the opinion that the activity was not due to prolactin itself. Using highly purified samples of prolactin, we found, on the contrary, that they show definite diabetogenic activity (see Table 1).

The same authors pointed out that estrone, unlike testosterone, produces a definite insulinotropic effect.[7] We were able to confirm, to a certain extent, and to enlarge this observation (see table). The synthetic estrogen, stilbestrol, shows even more insulinotropic activity than estradiol; and progesterone, and more particularly, testosterone, show diabetogenic effects.

A fact worthy of comment is that an insulinotropic effect has been obtained using such widely divergent substances as a protein fraction of the anterior pituitary on the one hand, and estradiol and stilbestrol on the other. The activity of the latter substances may perhaps be explained by stimulation of the anterior pituitary.

[1] We are indebted to the following: Dr. Erwin Schwenk, Schering Corporation, for estradiol, progesterone and testosterone; the U. S. Vitamin Corporation, N. Y., for stilbestrol; Professor H. M. Evans, Professor Abraham White and Dr. Oscar Riddle for samples of prolactin; and Dr. David Klein, Wilson Laboratories, for supplies of pituitary glands. We wish to thank Dr. Julius Rosenthal, director of the Pathological Laboratories of the Welfare Hospital, for his interest in our work.

[2] The "diabetogenic hormone" tends to increase the amount of sugar in the blood and tends to decrease the production of insulin in the pancreas. The "insulinotropic substance"—there is some debate as to whether we are dealing with a hormone—stimulates the production of insulin.

[3] C. H. Best, R. E. Haist and J. H. Ridout, *Jour. Physiol.*, 97: 107, 1939; J. Campbell and H. Keenan, *Canadian Chemical Process Industry*, 23, 280, 1939.

[4] H. P. Marks, cited in "Biological Standardization" by J. H. Burn, pp. 91, etc.

[5] According to Campbell and Keenan, fraction 2 should have contained D.H.

[6] H. P. Marks and F. G. Young, *Lancet* II, p. 710, 1940.

[7] See, also, E. Cantilo, *Endocrinology*, 28: 20, 1941, who describes the beneficial effects of estrogens in menopausal diabetes.

TABLE 1

Substance	Amount injected	No. of rats	Total weight of rats (in g.)	Insulin Un. per 100 g. body weight	Per cent. change
Anterior pituitary fractions:					
Control	...	10	2,900	0.36	...
Fraction 1	518 mg	10	2,200	0.64	+77
Fraction 2	180 mg	10	2,100	0.38	0
Fraction 3	1129 mg	10	2,050	0.26	−28
Fraction 4	63 mg	10	2,350	0.34	0
Prolactin preparations:					
Control	...	10	2,340	0.42	...
Evans (sheep) .	400 I.U.	10	2,740	0.00	−100
Evans (sheep) .	490 I.U.	7	1,550	0.02	− 96
White (ox) ...	300 I.U.	10	2,550	0.08	− 81
Riddle (ox) ...	300 I.U.	10	2,530	0.22	− 48
Sterols and stilbestrol:					
Control	...	10	2,080	0.43	...
Estradiol	12.6 mg	9	2,160	0.54	+19
Estradiol	14.0 mg	10	2,060	0.51	+19
Stilbestrol* ...	11.2 mg	8	1,710	0.67	+56
Stilbestrol* ...	14.0 mg	10	1,800	0.62	+44
Progesterone ..	12.6 mg	9	2,730	0.39	− 7
Progesterone ..	14.0 mg	10	2,690	0.36	−16
Testosterone ..	14.0 mg	10	2,350	0.29	−31
Testosterone ..	14.0 mg	10	2,870	0.27	−37

* The weight loss is due to toxicity.

SUMMARY

1. Insulinotropic effects were obtained with a protein fraction of the anterior pituitary, with estradiol and with stilbestrol.

2. Varying diabetogenic effects were obtained with highly purified prolactin preparations, with progesterone, with testosterone and with a fraction, probably also protein, from the anterior pituitary.

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CURE OF EGG-WHITE INJURY IN RATS BY THE "TOXIC" FRACTION (AVIDIN[1]) OF EGG WHITE GIVEN PARENTERALLY

RECENTLY[1] it has been shown, in experiments dealing with dietary egg-white injury, that raw or commercial egg white can be supplanted by a particular fraction of egg white (avidin). Previously[2] the specific biotin-binding capacity of this substance had been established by the yeast-growth method,[3] according to which biotin, in the presence of avidin, becomes unavailable for yeast cells and growth therefore ceases.

It has been found[1] that instead of the 20 to 30 per cent. of raw or commercial egg white needed in the experimental rations to produce egg-white injury in rats, from 0.03 to 0.07 per cent. of avidin is equally effective.

In view of these experimental results the presence of egg-white injury has been explained by the fixation of biotin to avidin in the intestine, the probable non-absorption of the avidin-biotin (AB) complex and its consequent excretion with the feces.

This assumption is borne out by experiments in which the presence of the avidin-biotin (AB) complex in the feces was directly tested. In the absence of avidin, biotin is present in tissues and in feces in free and in bound form, the latter being liberated only by intensive hydrolysis.[3] The avidin-biotin complex, on the other hand, is easily split by steaming for a short time (30 to 60 minutes) at 100 C. By the yeast-growth method no difference could be found in the biotin content of feces before and after steaming (4 to 5 micrograms per gram) when rats were fed a normal stock diet. By the same method it was found that rats fed a diet containing cooked egg white plus avidin, however, excreted only negligible amounts of free biotin (in one experiment 0.1 microgram per gram), whereas after the feces had been steamed large additional amounts of biotin became free (4.4 micrograms per gram in the experiment cited).[4]

In order to answer the question how avidin may act in parenteral administration a series of special experiments was required.

Concentrates of avidin were prepared[5] according to the procedure described by Eakin, Snell and Williams.[2] The biotin-binding capacity of each concentrate was tested quantitatively by the yeast-growth method.

To learn their effect on egg-white injury the avidin preparations were thoroughly mixed with pulverized, cooked dried egg white in varying amounts in different experiments which corresponded, on the basis of bio-

[1] The designation of the "toxic" protein of egg white tentatively proposed as *avidalbumin* (P. György, C. S. Rose, R. E. Eakin, E. E. Snell and R. J. Williams, SCIENCE, 93: 477, 1941) has been modified by the Texas group to *avidin*, because this protein does not entirely fit into the classification of an albumin. Additional details have now been published (R. E. Eakin, E. E. Snell and R. J. Williams, *Jour. Biol. Chem.*, 140: 535, 1941). In the present and previous studies avidin concentrates have been used, as pure crystallin avidin has not been available.

[2] R. E. Eakin, E. E. Snell and R. J. Williams, *Jour. Biol. Chem.*, 136: 801, 1940.

[3] E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Am. Chem. Soc.*, 62: 175, 1940.

[4] By variation of the normal diet and by feeding a pure meat ration we have so far been unable to detect in the feces of rats AB from which biotin could be liberated by steaming unless egg white was also present in the diet.

[5] The authors wish to express their thanks to Professor R. J. Williams and his collaborators, of the Department of Chemistry, University of Texas, for sending a generous supply of avidin concentrates. Other concentrates were prepared in the Laboratory of the Babies and Childrens Hospital of Cleveland.