Effect of Hypophysectomy on Glycogen Deposition in Isolated Muscle

RESULTS of experiments previously reported from this Institute in 1944 [1] indicated that when extracts of the anterior pituitary gland were administered to rats, insulin did not exhibit its usual action in promoting glycogen deposition in muscle as studied on the rat's diaphragm according to the technique of Gemmill[2]. In 1945 Cori *et al.*[3] reported the results of experiments on the effect of anterior pituitary extract and of insulin on hexokinase activity in tissue extracts obtained from rats.

It was found that when anterior pituitary extract was either injected into the intact animal or added to tissue extracts, hexokinase activity was inhibited. However, when insulin was added to such extracts, it appeared that this latter hormone released hexokinase from the anterior pituitary extract inhibition. Further, Cori considered that insulin *per se* did not enhance hexokinase activity.

As our investigations were carried out on intact muscle, it is difficult to make any strict comparison with the experiments of Cori.

It seemed, however, that any direct effect of insulin on hexokinase activity would be best investigated on hypophysectomized animals. Thus the inhibiting effect of the anterior pituitary on the hexokinase activity would be abolished, resulting in increase of glycogen deposition as compared with normal animals; and if a further increase occurred as a result of the addition of insulin it would be reasonable to assume that insulin exerted a direct action on hexokinase activity.

On the other hand, insulin should have no effect on the deposition of glycogen in the diaphragm of the hypophysectomized rats if its action were limited to the release of anterior pituitary inhibition.

In our experiments the methods employed have been reported in previous papers[1,4]. Hypophysectomy was performed according to the technique of Thompson[5].

Ten to fourteen days were allowed to elapse between the operation and excision of the diaphragm. A summary of the results obtained are shown in the following table.

Normal rats Mean of 28 animals			Hypophysectomized rats Mean of 20 animals		
Glycogen %			Glycogen %		
Without insulin (*a*)	With insulin (*b*)	Increase (*c*)	Without insulin (*d*)	With insulin (*e*)	Increase (*f*)
0·269	0·53	0·261	0·364	0·766	0·402

For columns (*a*) and (*d*) $t = 4{\cdot}19$ P 0·001
(*c*) and (*f*) $t = 5{\cdot}6$ P 0·001

From these results it appears that hypophysectomy increased the hexokinase activity in the isolated diaphragm of the rat and that it is further enhanced by the addition of insulin. Thus we are of the opinion that insulin has a direct effect on hexokinase activity.

We would like to express our sincerest thanks to Mr. A. F. Doutch for the statistical analysis.

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[1] Nelson, J. F., *Austral. J. Exp. Biol.*, **22**, 131 (1944).
[2] Gemmill, E., and Hamman, L., Jun., *Bull. Johns Hopkins Hosp.*, **68**, 50 (1941).
[3] Cori, C. F., Price, W. H., and Coldwick, S. P., *J. Biol. Chem.*, **160**, 633 (1945).
[4] Corkill, A. B., and Nelson, J. F., *Med. J. Aust.*, 172 (1) (1947).
[5] Thompson, D., *Endocrin.*, **16**, 257 (1932).

Cytochemical Demonstration of Ketosteroids

IN a number of cytochemical papers[1,2] it has been claimed that α-hydroxyketones, such as deoxycorticosterone, give a purple colour with reduced fuchsin in Feulgen's 'plasmal' reaction. In this reaction the material under test is treated with cold saturated aqueous mercuric chloride solution, then with reduced fuchsin. According to Dempsey *et al.*[3], aqueous mercuric chloride oxidizes the hydroxyketone to an aldehyde, which then reacts with reduced fuchsin.

We have now studied this reaction with three pure samples of deoxycorticosterone, one kindly supplied by Prof. T. Reichstein, Pharmazeutische Anstalt der Universitaet Basel, one kindly supplied by the British Drug Houses, Ltd., and the other prepared by one of us (C. W. S.) by hydrolysis of a sample of deoxycorticosterone acetate kindly supplied by Dr. F. T. Hewett, Organon Laboratories, Ltd., London, S.W.19. The sample prepared from the acetate was purified by sublimation at 0·001 mm. and recrystallized from ether.

With none of these samples have we been able to obtain any evidence of oxidation of deoxycorticosterone to an aldehyde by aqueous mercuric chloride.

We conclude that this technique cannot be relied upon to demonstrate the cytological location of α-hydroxyketones. Our experiments have been restricted to one such compound; but the facts of organic chemistry indicate that the α-hydroxyketo-grouping in deoxycorticosterone may be regarded as typical, and only atypical α-ketols, if any, will react as postulated by Dempsey and Wislocki.

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[1] Dempsey, E. W., and Bassett, D. L., *Endocrin.*, **33**, 384 (1943).
[2] Dempsey, E. W., and Wislocki, G. B., *Endocrin.*, **35**, 409 (1944); *Physiol. Rev.*, **26**, 1 (1946).
[3] Dempsey, E. W., *et al.* (private communication, Feb. 17, 1948).

p-Aminobenzoic Acid a Growth-Factor for Certain Brewer's Yeasts

MOST of the single-cell strains of English brewery top fermentation yeasts so far examined grow well at 25° C. in forty-eight hours in a synthetic glucose-mineral salts medium, containing $(NH_4)_2HPO_4$ as the sole source of nitrogen and biotin, pantothenic acid and inositol as accessory growth factors ('bios').

However, two single-cell strains, isolated from different breweries, failed to grow even after six days incubation at 25° C. in the medium containing the three factors mentioned above and in addition riboflavin, aneurin, nicotinic acid and pyridoxin. The missing factor proved to be *p*-aminobenzoic acid, addition of 0·01 μgm. per ml. of which induced heavy growth in the presence of other essential bios factors. One strain, designated 'Yeast 45', required biotin as an essential growth factor in addition to *p*-aminobenzoic acid, pantothenic acid (or β-alanine) being strongly stimulatory, but not essential for good growth. The other strain ('Yeast 47') required both biotin and pantothenic acid (or β-alanine) in addition