SUMMARY

1. The action of adrenal cortical extract (added *in vitro*) in causing diminution of glycogen breakdown in liver slices (rat and rabbit) has been confirmed.

2. It was shown that this inhibition is absent if the incubation of the liver slices is carried out in absence of oxygen. It is concluded that the active principle affects the synthesis rather than the breakdown of glycogen.

3. When total carbohydrate was estimated, a similar effect of the extract in preventing disappearance of the carbohydrate was observed. The effect is, therefore, not merely on the glycogen: glucose ratio.

4. Under conditions where carbohydrate synthesis can be demonstrated, there was always greater increase (whether estimated as glycogen or total carbohydrate) in the presence of the extract.

5. No effect was found on the adenosinetriphosphate level in the slices during carbohydrate synthesis in oxygen.

We are very grateful to Prof. D. Keilin, F.R.S., Dr E. F. Hartree and Prof. E. A. Doisy for gifts of glucose oxidase and catalase, and to Dr T. Mann for help in preparing glucose oxidase; we are greatly indebted to Messrs Parke, Davis and Co. for the supplies of eschatin free from preservative. Our thanks are due to the British Council for a studentship (C.Y.C.) and to the Medical Research Council for a personal grant (D.M.N.).

REFERENCES

- Bartlett, G. R., Wick, A. N. & MacKay, E. M. (1949). *J. biol. Chem.* **178**, 1003.
 Bendall, J. R. & Lehmann, H. (1941). *Nature, Lond.*, **148**, 538.
 Bendall, J. R. & Lehmann, H. (1942). *Biochem. J.* **36**, xiv.
 Chiu, C. Y. (1950). (In the Press.)
 Chiu, C. Y. & Needham, D. M. (1948). *Biochem. J.* **42**, xix.
 Cori, G. T., Swanson, M. A. & Cori, C. F. (1945). *Fed. Proc.* **4**, 234.
 Deane, H. W., Nesbitt, F. B., Buchanan, J. M. & Hastings, A. B. (1947). *J. cell. comp. Physiol.* **30**, 255.
 Dixon, M. & Elliott, K. A. C. (1929). *Biochem. J.* **23**, 812.
 Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
 Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
 Hartmann, F. A. (1942). *Endocrinology*, **30**, 861.
 Holmes, E. G. & Lehmann, H. (1940). *Brit. J. exp. Path.* **21**, 196.
 Keilin, D. & Hartree, E. F. (1948). *Biochem. J.* **42**, 221.
 Koepf, C. F., Horn, H. W., Gemmill, C. L. & Thorn, G. W. (1941). *Amer. J. Physiol.* **135**, 174.
 Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
 Lehmann, H. & Bendall, J. R. (1949). *1st Int. biochem. Congr. Abstr.* p. 624.
 Leupin, E. & Verzá, F. (1949). *Nature, Lond.*, **163**, 836.
 Long, C. N. H. (1942). *Endocrinology*, **30**, 870.
 Miller, B. F. & Van Slyke, D. D. (1936). *J. biol. Chem.* **114**, 583.
 Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
 Riesser, O. (1947). *Biochim. biophys. Acta*, **1**, 208.
 Russell, J. A. & Wilhelmi, A. E. (1941a). *J. biol. Chem.* **137**, 713.
 Russell, J. A. & Wilhelmi, A. E. (1941b). *J. biol. Chem.* **140**, 747.
 Seckel, J. P. G. (1940). *Endocrinology*, **26**, 97.
 Stadie, W. C. & Riggs, B. C. (1944). *J. biol. Chem.* **154**, 687.
 Stadie, W. C. & Zapp, J. A. (1947). *J. biol. Chem.* **170**, 55.
 Swingle, W. W. & Pfiffner, J. J. (1931). *Amer. J. Physiol.* **96**, 164.
 Van Wagtenonk, W. J., Simmons, D. H. & Hackett, P. L. (1946). *J. biol. Chem.* **163**, 301.
 Verzá, F. (1939). *Die Funktion der Nebennierenrinde*. Basel: Schwabe.
 Verzá, F. & Montigel, C. (1942). *Nature, Lond.*, **149**, 49.
 Verzá, F. & Wenner, V. (1948a). *Biochem. J.* **42**, 35.
 Verzá, F. & Wenner, V. (1948b). *Biochem. J.* **42**, 42.
 Verzá, F. & Wenner, V. (1948c). *Biochem. J.* **42**, 48.
 Villee, C. A. & Hastings, A. B. (1949). *J. biol. Chem.* **179**, 673.
 White, A. & Dougherty, F. T. (1947). *Endocrinology*, **41**, 230.

The Effect of Adrenal Cortical Preparations added *in vitro* upon the Carbohydrate Metabolism of Liver Slices

2. THE EFFECT OF SOME PURE STEROIDS UPON CARBOHYDRATE SYNTHESIS, OXYGEN UPTAKE AND NON-PROTEIN NITROGEN

By C. Y. CHIU, *Biochemical Laboratory, University of Cambridge*

(Received 15 August 1949)

After it had been shown that addition of adrenal cortical extract *in vitro* caused increased glycogen and total carbohydrate formation in liver slices (Chiu & Needham, 1950), it became of interest to find the effect of some pure adrenal cortical steroids. Deoxycorticosterone (DOC, as acetate and hydroxy succinate), 11-dehydrocorticosterone and 17-hy-

droxy-11-dehydrocorticosterone became available, and experiments are now described on their action, particularly in causing increased total carbohydrate synthesis. Some observations were also made on accompanying oxygen uptake and, in order to see whether participation of protein in the synthesis was indicated, on the changes in non-protein nitrogen.

EXPERIMENTAL

The preparation of the slices and the conditions of incubation were the same as described in the previous paper (Chiu & Needham, 1950).

Preparation of steroid solutions. The DOC acetate was dissolved in propylene glycol and water (2:1 by vol.); DOC hydrogen succinate was dissolved in the appropriate aqueous medium (Seckel's Ringer, Krebs's bicarbonate-Ringer or phosphate-Ringer) made slightly more alkaline (about pH 7.6). 11-Dehydrocorticosterone and 17-hydroxy-11-dehydrocorticosterone were sometimes dissolved in propylene glycol and water (2:1 by vol.); otherwise a solution was made in pure acetone, this was diluted with water to the required concentration and the acetone was driven off by a current of air. The amount used varied between 12.5 and 25 $\mu\text{g./ml.}$ medium.

Chemical methods. The methods for glycogen, total carbohydrate and glucose were the same as previously described (Chiu & Needham, 1950).

O_2 uptake was measured with Warburg manometers using phosphate-Ringer as suspension medium. For non-protein nitrogen (NPN) determination, 1 ml. 10% (w/v) Na tungstate and 1 ml. 0.66 N- H_2SO_4 were added to the contents of each flask. A measured portion of the filtrate was used for soluble N estimation by a modification of Pregl's micro-Kjeldahl method (Harrison, 1947). Duplicate samples were used for each determination and the average difference was ± 2.7 , extreme limits $\pm 5.4\%$.

Urea was estimated manometrically by the method of Krebs & Henseleit (1932), and lactic acid by the micro-method of Barker & Summerson (1941).

RESULTS

The results on carbohydrate synthesis are summarized in Figs. 1-3.

It should be mentioned that when the steroid was used dissolved in propylene glycol (experiments of Figs. 1 and 2), an equal amount of this solvent was added to the control incubated without steroid. It was found that the solvent in the amount used (0.1 ml./2 ml. medium) had no effect on the final glycogen content; slightly larger amounts, however (0.3 ml./2 ml. medium), had a marked effect in decreasing the final content.

In every case, when glycogen or total carbohydrate disappeared, this disappearance was less in presence of steroid; when synthesis took place, the synthesis was greater in presence of steroid.

With regard to comparison of the behaviour of the three steroids, the following facts may be noted: (a) In the experiments done with added substrate (pyruvate) with an atmosphere of oxygen (Fig. 2) 17-hydroxy-11-dehydrocorticosterone was more effective than DOC acetate. Dehydrocorticosterone is about as effective as DOC acetate. (b) In the experiments with slices from well-fed livers, done without added substrate and with an atmosphere of air, DOC and dehydrocorticosterone were again about equally effective, but here 17-hydroxy-11-de-

hydrocorticosterone had definitely less effect than either of the other compounds. In these experiments the DOC was used in the form of succinate, and all the steroids were dissolved in aqueous medium.

Fig. 1. Glycogen changes in rat- and rabbit-liver slices with and without addition of DOC acetate. Animals all fasted, rats 24 hr., rabbits 48 hr. Incubation, 1 hr. at 37° . Medium: Seckel's Ringer for nos. 1 and 2; Krebs's Ringer for nos. 3-10; phosphate-Ringer for nos. 11 and 12. Gas phase: O_2 or 95% O_2 -5% CO_2 (according to medium). Substrate for experiments with rat tissue was 0.4% pyruvate, with rabbit tissue 1% glucose. DOC acetate dissolved in propylene glycol and water (2:1 by vol.) and equivalent amount of solvent added to the control. Addendum: 25 $\mu\text{g.}$ of steroid in 0.1 ml. added to 2 ml. medium. Unshaded = initial value; black = value after incubation with substrate + DOC; shaded = value after incubation with substrate only.

In some of the experiments of Fig. 3 without added substrate, particularly with high initial glycogen and of short duration, a considerable increase in total carbohydrate above the initial is seen; in others, on the other hand, there is a marked disappearance of carbohydrate, although this disappearance is less when the hormone is present. The products of this glycogen breakdown have not yet been investigated. Probably they include lactic acid and some intermediate stages in glycolysis as well as oxidation products. Possibly gluconeogenesis can most readily be seen at an early stage, breakdown following at a later stage. A few experiments in which lactic acid formation was estimated showed no significant effect of the steroids on the amount formed.

Fig. 4 shows the results of measurements of oxygen uptake with and without steroid. It will be seen that in all experiments except one there is little, if any, effect of the steroid.

Not much work was done with regard to urea formation, since it was found that the initial value for urea was very variable, however carefully sampling was done; for instance, in one experiment,

Fig. 2. Total carbohydrate changes in rat-liver slices with and without pure steroids. All animals fasted 24 hr. Incubation, 1 hr. at 37° (except nos. 6 and 7, 2 hr.). Medium: phosphate-Ringer. Gas phase: O₂. Substrate: 0.4% pyruvate. Steroids all dissolved in propylene glycol and water (2:1 by vol.) and equivalent amount of solvent added to the control. Addendum, 25 µg. of steroid in 0.1 ml. added to 2 ml. medium. DOCA=deoxycorticosterone acetate. DHC=11-dehydrocorticosterone. 17-OH-DHC=17-hydroxy-11-dehydrocorticosterone. Method (b) (see text) was used for the carbohydrate estimations. Unshaded=initial value; black=value after incubation with substrate + steroid; shaded=value after incubation with substrate only.

Fig. 3. Total carbohydrate changes in rat- and rabbit-liver slices, with and without pure steroids. All animals were fed except no. 16. No substrate added. Incubation, 1 hr. at 37° for all except 5-10; for these, 20 min. at 37°. Gas phase; air. Medium: phosphate-Ringer for nos. 1-3, 13 and 16; Krebs's Ringer for nos. 4, 11, 12, 14 and 15; Seckel's Ringer for nos. 5-10 and 17. Steroids all in aqueous solution. Addendum: 50 µg. in 2 ml. medium. DOCS=deoxycorticosterone succinate; DHC=11-dehydrocorticosterone; 17-OH-DHC=17-hydroxy-11-dehydrocorticosterone. Method (a) was used for nos. 11-15; method (b) for nos. 4-10 and 16; method (c) for nos. 1-3 and 17 (see text). Unshaded=initial value; black=value after incubation with substrate + steroid. Shaded=value after incubation with substrate only.

determinations in triplicate for initial urea gave 26.4, 37.2 and 19.4 $\mu\text{g.}/100\text{ mg.}$ wet tissue. When the

Fig. 4. Total carbohydrate synthesis and O_2 uptake in rat-liver slices with and without steroid. Animals nos. 1-3, well fed; nos. 4-6, fasted. Incubation 1 hr. at 37° . Medium: phosphate-Ringer. Gas phase: nos. 1-3, air; nos. 4-6, O_2 . Substrate: nos. 1-3, none added; nos. 4-6, 0.4 % pyruvate. Steroid added: 50 $\mu\text{g.}$ in 2 ml. medium for nos. 1-3; 25 $\mu\text{g.}$ in 2 ml. medium for nos. 4-6. DOCA = deoxycorticosterone acetate; DOCS = deoxycorticosterone succinate; DHC = 11-dehydrocorticosterone; 17-OH - DHC = 17 - hydroxy - 11 - dehydrocorticosterone. Method (b) was used for total carbohydrate estimation for nos. 4-6, method (c) for nos. 1-3 (see text). Black = value of O_2 uptake after incubation with steroid; shaded = value of O_2 uptake after incubation without steroid; cross-hatched = value of total carbohydrate in presence of steroid minus the value in absence of steroid.

slices were washed by immersing twice in 5 ml. incubation medium, the initial value fell to 0, but with

such washed slices, in preliminary experiments, there was little glycogen formation from glutamate, and this question was not further investigated. These experiments did, however, show that there was no striking effect of the steroid on urea formation. Thus in one experiment without steroid the value was 23.5 $\mu\text{g.}/100\text{ mg.}$ tissue, with eschatin 23.4; and in a second, 13 $\mu\text{g.}$ without hormone and 8 $\mu\text{g.}$ with hormone.

The experiments done on non-protein nitrogen are summarized in Table 1. It is to be noticed that: (a) in all cases but one there is slightly more non-protein nitrogen present after incubation with steroid than without; (b) the total amount of non-protein nitrogen present, even in the samples with steroid, seems small if one is to suppose that the extra carbohydrate is being formed at the expense of protein or of substances in the non-protein nitrogen fraction. Awapara, Marvin & Wells (1949) recently found small amounts (about 30 $\mu\text{g.}/100\text{ mg.}$ tissue) of free amino-acid nitrogen in liver. The amino-acids concerned were dicarboxylic acids, alanine and glycine, together with traces of others. The balance of the amino-acids was changed in livers from adrenalectomized animals, the dicarboxylic acids being present in higher proportion. Restoration of normal balance took place after injection of the adrenalectomized animals with adrenal cortical extract, and this was taken as an indication that the dicarboxylic amino-acids might be used preferentially for carbohydrate formation.

DISCUSSION

It is interesting to notice in Fig. 4 that there is no correlation between oxygen uptake and extra carbohydrate formation as a result of the presence of the

Table 1. *Effect of eschatin and pure steroids on non-protein nitrogen of liver slices*

(About 400 mg. slices were suspended in 3 ml. phosphate-Ringer for 1 hr. at 37° . Gas phase: O_2 . Dose: eschatin, 0.15 ml./ml. medium; steroid, 25 $\mu\text{g.}/\text{ml.}$ medium. —, not estimated.)

Date	Animal	Extract or pure steroid added	Increase in total carbohydrate in sample + steroid above control ($\mu\text{g.}/100\text{ mg.}$ tissue)	Non-protein nitrogen ($\mu\text{g.}/100\text{ mg.}$ tissue)			
				Initial (a)	Final, control (b)	Final, with extract or steroid (c)	Difference (c-b)
June 1947	Rabbit, fasted	Eschatin	50	—	337	335	-2
July 1947	Rabbit, fasted	Eschatin	385	—	235	322	+87
Aug. 1947	Rabbit, fasted	Eschatin	133	—	96	107	+11
Aug. 1947	Rabbit, fasted	Eschatin	52	—	83	102	+19
Aug. 1947	Rabbit, fasted	Eschatin	175	—	96	118	+22
Mar. 1949	Rabbit, non-fasted	Eschatin	—	128	140	147	+7
Mar. 1949	Rabbit, fasted	17-Hydroxy-11-dehydrocorticosterone	331	121	78	92	+14
May 1949	Rat, well fed	DOC succinate	656	—	161	184	+23
May 1949	Rat, well fed	DOC succinate	663	—	150	169	+19

steroid. Koepf, Horn, Gemmill & Thorn (1941) observed similarly (using liver slices from normal and adrenalectomized rats and rats adrenalectomized and injected with adrenal cortical hormone) that there was no parallelism between oxygen uptake and power to synthesize carbohydrate. Bartlett, Wick & MacKay (1949) also found with diaphragm that glycogen formation could be increased by insulin or decreased by deoxycorticosterone without affecting oxygen uptake.

From experiments on the whole animal, DOC has usually been associated with influence on mineral metabolism while the steroids with oxygen at C_{11} are associated with control over carbohydrate metabolism. In these experiments *in vitro*, however, we found DOC to be just as potent as 11-dehydrocorticosterone in increasing carbohydrate formation. It has to be remembered that even *in vivo* there is no hard and fast distinction between the steroids controlling mineral metabolism and those controlling carbohydrate metabolism: thus Harrison & Harrison (1939) showed that daily injection of 1.25 mg. deoxycorticosterone was enough to maintain normal potassium:sodium balance in the serum of rats, while injection of 2.5 mg. maintained normal blood sugar. Young (1944) has pointed out that in experiments *in vivo* where water-insoluble DOC compounds are used, rate of absorption may play a part.

SUMMARY

1. The effect of the three pure adrenal cortical steroids, deoxycorticosterone, 11-dehydrocorticosterone and 17-hydroxy-11-dehydrocorticosterone, when added *in vitro*, upon carbohydrate synthesis in liver slices was investigated.

2. In all cases, whether glycogen or total carbohydrate content was estimated, there was greater increase in carbohydrate content or less carbohydrate disappearance in presence of the steroid than in the controls without added steroid.

3. The presence of the steroids had little or no effect on oxygen uptake.

4. In preliminary experiments, slightly increased concentration of non-protein nitrogen was found in presence of eschatin or of the pure steroids.

I wish to express my hearty gratitude to Dr D. M. Needham, F.R.S., for her untiring advice and continuous help throughout the course of this work.

The pure steroids used were gifts to Dr D. M. Needham and myself: the DOC acetate from Messrs Organon and from Dr Tissières; the DOC succinate from Dr Wettstein of Messrs Ciba; the 11-dehydrocorticosterone from Messrs Organon and the 17-hydroxy-11-dehydrocorticosterone from Dr T. Reichstein. For all these we express our thanks.

I am greatly indebted to Prof. A. C. Chibnall, F.R.S., for the hospitality of his laboratory and to the British Council for a 3-year grant.

REFERENCES

- Awapara, J., Marvin, H. N. & Wells, B. B. (1949). *Endocrinology*, **44**, 378.
 Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
 Bartlett, G. R., Wick, A. N. & MacKay, E. M. (1949). *J. biol. Chem.* **178**, 1003.
 Chiu, C. Y. & Needham, D. M. (1950). *Biochem. J.* **46**, 114.
 Harrison, G. A. (1947). *Chemical Methods in Clinical Medicine*, 3rd ed. London: Churchill.
 Harrison, H. E. & Harrison, H. C. (1939). *Proc. Soc. exp. Biol.*, N.Y., **42**, 506.
 Koepf, C. F., Horn, H. W., Gemmill, G. L. & Thorn, G. W. (1941). *Amer. J. Physiol.* **135**, 174.
 Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
 Young, F. G. (1944). *Ann. Rev. Physiol.* **6**, 427.

Studies in Detoxication

29. THE ORIENTATION OF GLUCURONIC ACID CONJUGATION IN CHLOROQUINOL

By K. S. DODGSON, J. N. SMITH AND R. T. WILLIAMS

Physiology Institute, Cardiff, and Department of Biochemistry, St Mary's Hospital Medical School, London

(Received 26 August 1949)

Dodgson & Williams (1949) have shown that 4-chlorocatechol and 4-chlororesorcinol form, in rabbits, monoglucuronides (I and II) in which the glucuronic acid residue is attached to the hydroxyl group farthest away from the chlorine atom. On these grounds it was predicted that chloroquinol would give rise in the rabbit to the monoglucuronide (III), in which the glucuronic acid is attached to the hydroxyl *meta* to the chloro group.

In this paper we shall prove that the glucuronide of chloroquinol synthesized in the rabbit has, in fact, structure (III), i.e. that of 3-chloro-4-hydroxy-