STUDIES ON THE ROLE OF THE ADRENAL CORTEX IN ADENOSINE TRIPHOSPHATE SHOCK.

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SINCE Selye (1936*a*, *b*) called attention to the adrenal changes following exposure of the body to noxious agents, an ever-increasing volume of work on this subject (Selye, 1946 ; Long, 1947 ; Sayers and Sayers, 1948) has led to the development of a theory, now firmly established, that injury to the body, in any form, stimulates the hypophysis to liberate adrenocorticotrophic hormone (ACTH), which excites the secretion of adrenal cortical hormone mainly of the gluco-corticoid type. In previous work (Green, 1943 ; Bielschowsky and Green, 1943 ; Green and Stoner, 1950*a*) we have shown that adenosine triphosphate (ATP) will produce a state of shock in animals almost indistinguishable from that seen after limb ischaemia. One of the essential steps in this work was to study the behaviour of the adrenal cortex in ATP shock. The effect of ATP on the adrenal cortex has more than a passing interest, since it is not a foreign toxic agent like those usually used to induce an alarm reaction, e.g. formaldehyde, but a normal constituent of the body which may play an important part in the response to injury (Green and Stoner, 1950*a*, *b*). It has also been advocated in the treatment of rheumatoid arthritis (Lövgren, 1945), although this claim has not been confirmed (Wayne, Goodwin and Stoner, 1949).

The present study had the twofold object of determining the role of the adrenal hormones in resistance to the shock-inducing action of ATP, and the effect of ATP on the functional activity of the gland as judged by changes in its histochemistry. Whilst the precise significance of the individual histochemical tests used is not clear (Bennett, 1940 ; Weaver and Nelson, 1943 ; Popják, 1944 ; Albert and Leblond, 1946 ; Harrison and Cain, 1947 ; Yoffey and Baxter, 1947, 1949 ; Boscott and Mandl, 1949 ; Greep and Deane, 1949*a* ; Cain, 1950), by using several tests simultaneously it is possible to obtain a picture of the activity of the adrenal cortex (Dempsey and Wislocki, 1946). Furthermore, since the various zones of the adrenal cortex are separately controlled and secrete different hormones, the histochemical method possesses advantages over a purely chemical approach.

METHODS.

Three hundred and twenty albino rats, male and female, from one source were used. The sexes were used separately and not mixed in any one experiment. All operations were carried out under ether anaesthesia. Bilateral adrenalectomy was performed in one stage by the dorsal route. These animals were given 1 per cent NaCl to drink after operation, and used 3 days after removal of the

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glands. Adrenal medullectomy was done by the method of Ingle and Griffith (1942). These rats were given 1 per cent NaCl to drink for 1 week after the operation and used at the end of 3 weeks, unless stated otherwise. The completeness of the medullectomy was checked histologically at the end of the experiment. Hypophysectomy was performed by the parapharyngeal route and the rats used 3 days after the operation. The completeness of the hypophysectomy was determined at autopsy.

Ischaemic shock was produced in a few rats by applying rubber tourniquets to both hind limbs under ether anaesthesia (Rosenthal, 1943).

ATP was obtained as the Ba salt (Boots Pure Drug Co.) and converted to the Mg salt for use (Green and Stoner, 1950*a*). Paper chromatograms were made by Mr. C. J. Threlfall with this and also with muscle adenylic acid (A5MP ; Boots Pure Drug Co.), and adenosine (British Drug Houses Ltd.). These showed that the ATP contained approximately 10 per cent A5MP, whilst the other substances appeared to be pure. The control rats were given D-ribose routinely in amount and concentration equivalent to that contained in the nucleotide or nucleoside injected. A few animals were given equivalent volumes of 0·9 per cent NaCl for comparison with the ribose controls. The solutions were injected intraperitoneally at body temperature (Long, 1947), and with as little disturbance to the animal as possible. Of the cortical hormones desoxycorticosterone was used as the acetate and glucoside (Ciba Ltd.), and " Eucortone " (Allen & Hanbury, Ltd.) was used as a whole gland extract. Our thanks are due to Dr. Tindall, of Organon Ltd., for supplying us with samples of ACTH.

Toxicity tests were done on male rats fasted overnight, care being taken to ensure a constant environmental temperature during the test (Green and Stoner, 1950*a*). Rectal temperatures were recorded with a thermocouple.

Rats used for histochemical studies were killed by a blow on the head and bled from the neck. The adrenals were rapidly removed and weighed. Ascorbic acid was detected by Deane and Morse's modification (1948) of Barnett and Bourne's method (1941). Glands fixed for 48 hours in 10 per cent formol-saline and then washed in running tap water for 24 hours were used to demonstrate ketones (50μ frozen sections) by the method of Albert and Leblond (1946), and frozen sections (20μ) from the same material were examined between crossed nicol prisms or polaroid discs for the presence of birefringent material. Birefringence was also studied in frozen sections from glands fixed for 5 days at 37° C. in Bennett's (1940) formol-digitonin solution. The acetone solubility of such material was checked on several occasions. Frozen sections (20μ) from glands fixed by both methods were stained for sudanophilic lipid with Sudan III.

Plasma Mg concentrations were determined by Garner's method (1946) on samples of heart blood obtained under ether anaesthesia using heparin as an anticoagulant.

RESULTS.

Effect of adrenal hormones on the shock-inducing action of ATP.

Sensitivity to the shock-inducing action of ATP was greatly increased by adrenalectomy. The difficulties in determining the lethal dose of ATP have been discussed before (Green and Stoner, 1950*a*). In young rats of this particular strain, and under our environmental conditions (air temp. 22° C.) the LD_{100}

proved to be very high, and was between 150 to 200 mg. MgATP per 100 g. body weight (i.p.). Young animals are more resistant than older ones (Green and Stoner, 1950*a*), and this was well borne out in these experiments. After adrenalectomy the lethal dose was 10 mg. per 100 g. body weight (i.p.), and variations in toxicity due to age appeared to be eliminated. In the normal rat after a fatal dose of ATP the body temperature falls rapidly to a level close to the air temperature, where it remains for some time before death occurs (Green and Stoner, 1950*a*). Doses of ATP, without effect in the normal animal, lowered the body temperature after adrenalectomy. However, death usually occurred at a higher body temperature in the adrenalectomized rat than in the normal rat, probably because adrenalectomy decreased both the fatal dose and the survival time after such a dose. The rate of fall of the body temperature after ATP was about the same in the normal and adrenalectomized animal.

The results of toxicity tests in medullectomized rats were rather variable, but sufficient were done to show that sensitivity to the shock-inducing action of ATP was increased after this operation. Such animals often died after doses of 80 mg. MgATP per 100 g. body weight which were never fatal in the present series of normal rats, and if they survived such doses their clinical state was always worse than that of control normal rats. Hypophysectomy led to a similar, but less marked, increase in sensitivity.

Attempts to restore the resistance of the adrenalectomized rat by giving adrenal cortical hormones were not very successful. Giving 3 mg. desoxycorticosterone acetate per day (i.m.) for the 3 days after removal of the glands and 5 mg. of the glucoside (i.m.) just before injecting the ATP only raised the lethal dose to 40 mg. MgATP per 100 g. body weight. A similar increase could be obtained by giving 1·0 ml. doses of " Eucortone " in the same way, and continuing them at hourly intervals for 3 hours after the ATP injection.

Selye (1949) has described an antagonism between the mineralo-corticoids and the gluco-corticoids of the adrenal cortex, and states that the response to various stress-inducing agents is increased by the former and reduced by the latter. Whilst we found a slightly increased response to limb ischaemia after pre-treatment with large doses of desoxycorticosterone, this substance appeared to have little effect on the response to ATP. It was given either in daily doses of 3 mg. of the acetate (i.m.) for 4 days followed by a single dose of 5 mg. of the glucoside just before the experiment, or as 3-mg. doses of the acetate (i.m.) just before and 4 hours after the injection of the ATP, and in neither case was the response significantly altered. " Eucortone " had a slight therapeutic action. When given in 1·0 ml. per 100 g. body weight doses (i.m.) just before and again at 2 and 4 hours after the injection of ATP it improved the clinical state of the animal whilst not affecting the mortality rate. This effect has also been seen in mice.

Effect of ATP on the histochemistry of the adrenal cortex.

In most experiments a dose of ATP (75 to 100 mg. MgATP per 100 g. body weight) was chosen which would give a moderately severe, but non-fatal, state of shock as judged by the body temperature (Fig. 1). The combined adrenal weight was determined in 66 male rats killed at varying intervals up to 24 hours after this dose, and compared with that in 52 male rats killed at similar intervals

after the injection of an equivalent amount of D-ribose. D-ribose caused no apparent disturbance in the animals, and when their combined adrenal weights, expressed as mg. per 100 g. body weight, were plotted against the body weight, the points fell on a curve similar to that shown by Andersen and Kennedy (1932), illustrating the reciprocal relationship between adrenal weight and body weight. The combined adrenal weights of the ATP-treated rats, expressed in the same way, fell about the same curve.

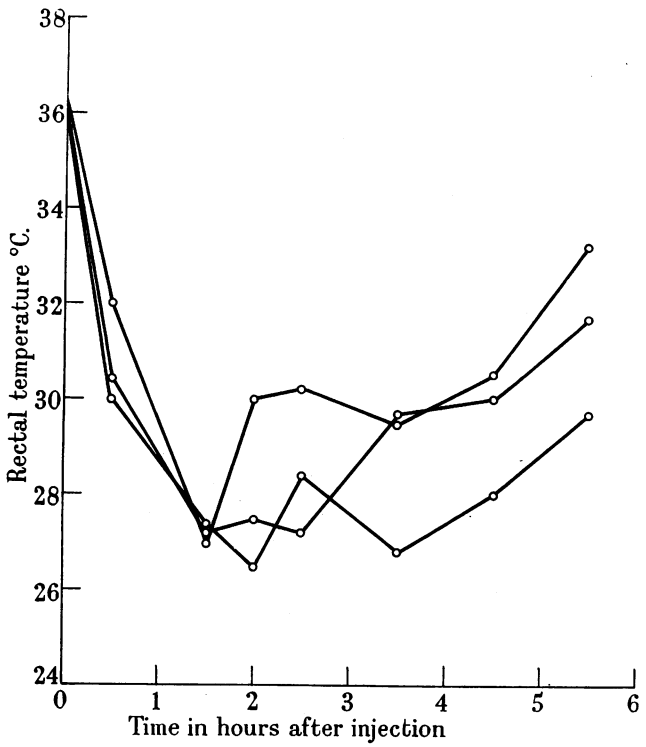


FIG. 1.—The effect of non-lethal doses of ATP on the rectal temperature. Each curve shows the changes in the rectal temperature of a fasting rat given 75 mg. MgATP per 100 g. body weight (i.p.) at zero time.

In the haematoxylin and eosin counterstained paraffin sections from the glands stained for ascorbic acid the adrenal cortex appeared hyperaemic after ATP. There was also an increase in the size of the cells of the zona fasciculata, which was maximal 7 hours after the injection (Fig. 3 and 4). This increase in cell size is of interest in view of the absence of any overall change in weight and the loss of much of the contents of these cells (v.i.). The change is probably due to an increase in their water content (Popják, 1944). The average water content of the whole adrenal gland 7 hours after this dose of ATP was 74·3 per cent, which was slightly higher than in the controls (73·6 per cent), but the difference was not statistically significant. Estimations of the fat free dry weight (Hastings and Eichelberger, 1937) showed that there was a significant decrease in the fat content of the adrenal at this time after the ATP injection.

The number and distribution of the rats receiving a non-fatal dose of ATP whose adrenals were examined histochemically is given in Table I.

TABLE I.—*Shows the Number of Rat Adrenals Examined Histochemically at Different Intervals after the Injection of a Non-fatal Dose of ATP.*

Time (hr.) after i.p. non-fatal dose of ATP (exp.) or an equivalent amount of d-ribose (control).	Histochemical test.															
	Ascorbic acid.				Sudanophilia.				Birefringence.						Ketones.	
									With digitonin.				Without digitonin.			
	Exp.		Control		Exp.		Control.		Exp.		Contr.		Exp.	Contr.	Exp.	Con.
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1	5	4	3	4	6	1	5	1	6	1	4	1	3	2	4	2
4	5	2	3	2	18	2	6	2	9	2	3	2	8	5	6	4
7	3	–	3	–	6	–	4	–	5	–	4	–	3	2	2	2
24	3	3	3	3	5	3	3	3	5	3	3	3	–	–	1	1

After a non-fatal dose of ATP the first histochemical change in the adrenal cortex was a fall in its ascorbic acid content. Control rats, given D-ribose, showed numerous argentophil granules spread uniformly in the cytoplasm, being densest in the cells of the zona fasciculata (Fig. 2). There were few granules in the cells of the zona glomerulosa and in the zona reticularis, their number diminished as the medulla was approached. The picture in these rats remained the same throughout the 24-hour period studied, and closely resembled that given for the normal rat by Deane and Morse (1948). After ATP the number and size of the granules in the cells of the zona fasciculata decreased progressively, reaching a minimum 7 hours after the injection (Fig. 3 and 4). Argentophil granules are normally present between the cells in the sinusoids, indicating an early stage in secretion (Deane and Morse, 1948). This was very prominent in glands examined 1 and 4 hours after the injection of ATP (Fig. 5). The ascorbic acid content of the zona reticularis fell, but the granules in the zona glomerulosa were unaffected by ATP. By 24 hours normal conditions had been restored, and there was now no difference between the treated and control animals.

Lipid distribution in the normal rat adrenal cortex is rather variable (Harrison and Cain, 1947 ; Cain and Harrison, 1950). In our control material one usually saw a densely packed zona glomerulosa, separated by a narrow, relatively sudanophobic zone from the fairly uniformly dense zonae fasciculata and reticularis where the density of lipid decreased near the medulla (Fig. 6). After ATP definite changes in the amount of sudanophilic material were not seen till 4 hours after the injection. In glands taken at 4 hours (Fig. 7 and 8) and 7 hours there was always considerably less than in the control glands. The loss of lipid was confined to zonae fasciculata and reticularis, where the change was comparable to that produced by the injection of between 1 and 3 mg. ACTH (Fig. 9). Complete recovery occurred in 24 hours.

The phenylhydrazone-forming material of the normal adrenal cortex is concentrated chiefly in the zona glomerulosa and outer part of the zona fasciculata. After ATP the ketone material in the zona fasciculata was reduced, whilst that in the zona glomerulosa remained unchanged. The time relations of these changes were the same as for the sudanophilic material.

EXPLANATION OF PLATES.

PLATE I (Fig. 2–5).

High power (× 700) photomicrographs of the zona fasciculata of the rat adrenal cortex after treatment with $AgNO_3$ to demonstrate ascorbic acid.

FIG. 2.—Rat (142 g. body weight, ♂) killed 1 hour after the injection of a dose of D-ribose equivalent to 75 mg. MgATP per 100 g. body weight. The section shows the normal distribution of the argentophil granules in the cytoplasm.

FIG. 3.—Rat (124 g. body weight, ♂) killed 1 hour after the injection (i.p.) of 90 mg. MgATP per 100 g. body weight.

FIG. 4.—Rat (134 g. body weight, ♂) killed 7 hours after the injection (i.p.) of 75 mg. MgATP per 100 g. body weight.

FIG. 3 and 4 show the reduction in the number and size of the granules and the increase in the size of the cells after ATP.

FIG. 5.—Rat (178 g. body weight, ♀) killed 1 hour after the injection (i.p.) of 75 mg. MgATP per 100 g. body weight. The granules are seen to be lying in the sinusoids between the cells showing an early stage in secretion.

PLATE II (Fig. 6–9).

Low-power (× 75) photomicrographs of the male rat adrenal cortex treated with Sudan III.

FIG. 6.—Rat (116 g. body weight) killed 4 hours after the injection (i.p.) of a dose of D-ribose equivalent to 95 mg. MgATP per 100 g. body weight. The section shows the normal distribution of sudanophilic lipid in the zones of the cortex.

FIG. 7.—Rat (139 g. body weight) killed 4 hours after the injection (i.p.) of 75 mg. MgATP per 100 g. body weight.

FIG. 8.—Rat (154 g. body weight) killed 4 hours after the injection (i.p.) of 75 mg. MgATP per 100 g. body weight.

FIGS. 7 and 8 show the decrease in the sudanophilia of the zonae fasciculata and reticularis after ATP, with the retention of lipid in the zona glomerulosa.

FIG. 9.—(a) Rat (95 g. body weight) killed 4 hours after the injection (i.p.) of 3 mg. ACTH. (b) Rat (110 g. body weight) killed 7 hours after the injection (s.c.) of 3 mg. ACTH.

The sections show the discharge of sudanophilic lipid from the zonae fasciculata and reticularis.

PLATE III (Fig. 10).

Low-power (× 70) photomicrographs of the male rat adrenal cortex fixed in formol-digitonin and examined between crossed polaroid discs. The white line indicates the position of the capsule.

The lower series is from rats given 75 mg. MgATP per 100 g. body weight (i.p.) and the upper from rats given an equivalent amount of D-ribose. The numbers on the photographs show the interval (hours) between the injection and the killing of the animal.

The sections show the great decrease in the birefringence of the zona fasciculata after ATP administration. The apparent changes in the zona glomerulosa are artefacts (see text).

PLATE IV (Fig. 11 and 12).

FIG. 11.—Low power (× 70) photomicrograph of the male rat adrenal cortex fixed in formol-digitonin. Rat (110 mg. body weight) killed 7 hours after the injection (s.c.) of 3 mg. ACTH. The white line indicates the position of the capsule. This section should be compared with those in Fig. 10 taken from ATP-treated rats.

FIG. 12.—Low-power (× 75) photomicrographs of the male rat adrenal cortex treated with Sudan III. The sections show the increase in the sudanophilic lipid of the cortex after repeated treatment with ATP (b) as compared with the normal density of the control (a). See text for details.

PLATE V (Fig. 13).

Photomicrographs of the adrenal cortex from a female hypophysectomized rat which died 4¼ hours after the injection (i.p.) of 110 mg. MgATP per 100 g. body weight.

FIG. 13a.—Fixed in 10 per cent formol-saline and treated with Sudan III. (× 75.)

FIG. 13b.—Fixed in formol-digitonin and examined, unstained, between crossed polaroid discs (× 70). The white line indicates the position of the capsule.

The sections show the retention of sudanophilic lipid and birefringent material after the injection of ATP in the hypophysectomized animal.

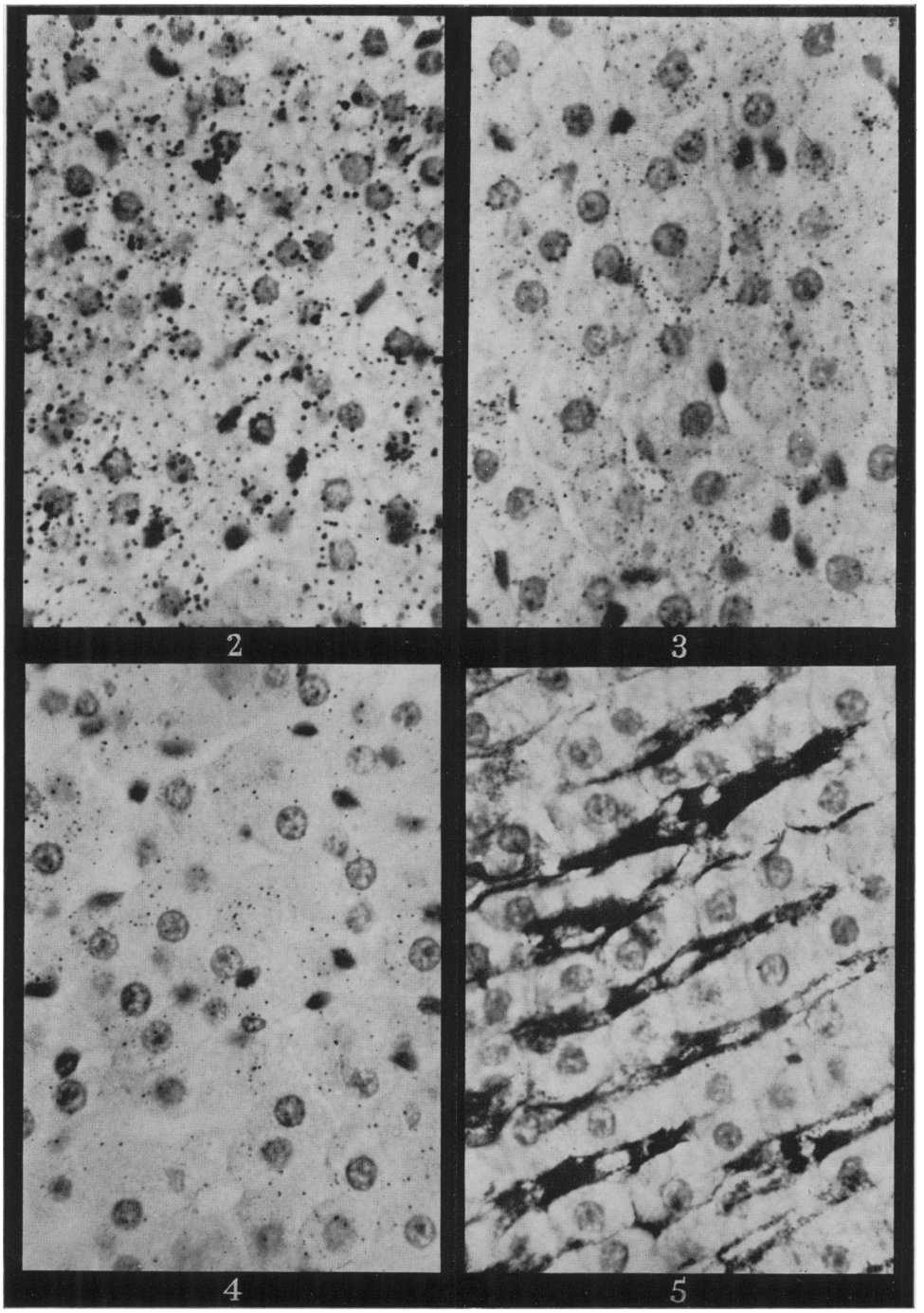
PLATE VI (Fig. 14).

Photomicrographs of the male rat adrenal 3 weeks after medullectomy, and fixed in 10 per cent formol-saline.

FIG. 14a.—Rat (148 g. body weight) which died 3¼ hours after the injection (i.p.) of 150 mg. MgATP per 100 g. body weight. Treated with Sudan III. (× 75.)

FIG. 14b.—Rat (152 g. body weight) which died 6 hours after the injection (i.p.) of 90 mg. MgATP per 100 g. body weight. Examined unstained between crossed polaroid discs (× 70). The white line indicates the position of the capsule.

The sections show the persistence of the sudanophilic lipid and birefringent material after ATP in the adrenal medullectomized rat.



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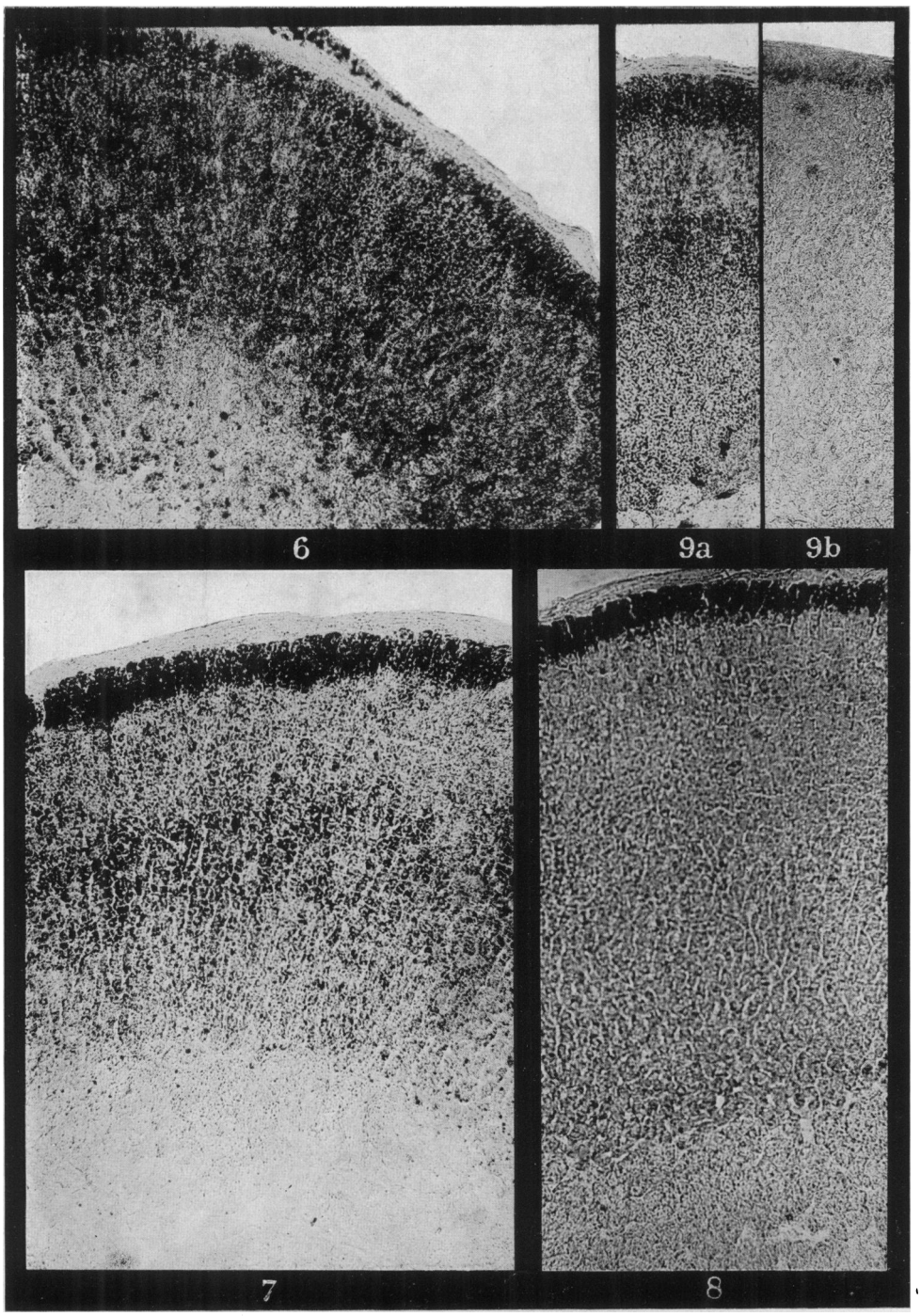

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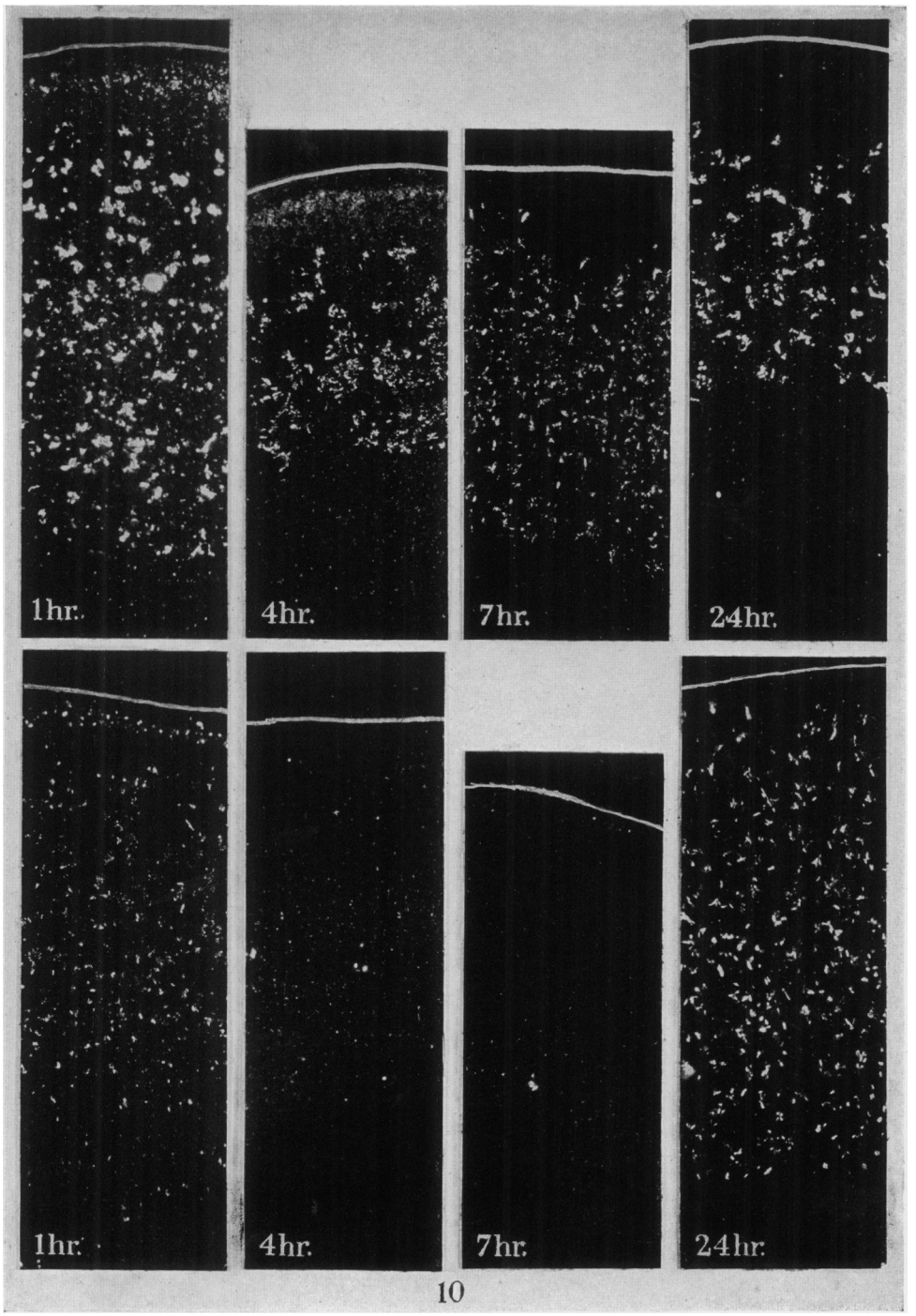

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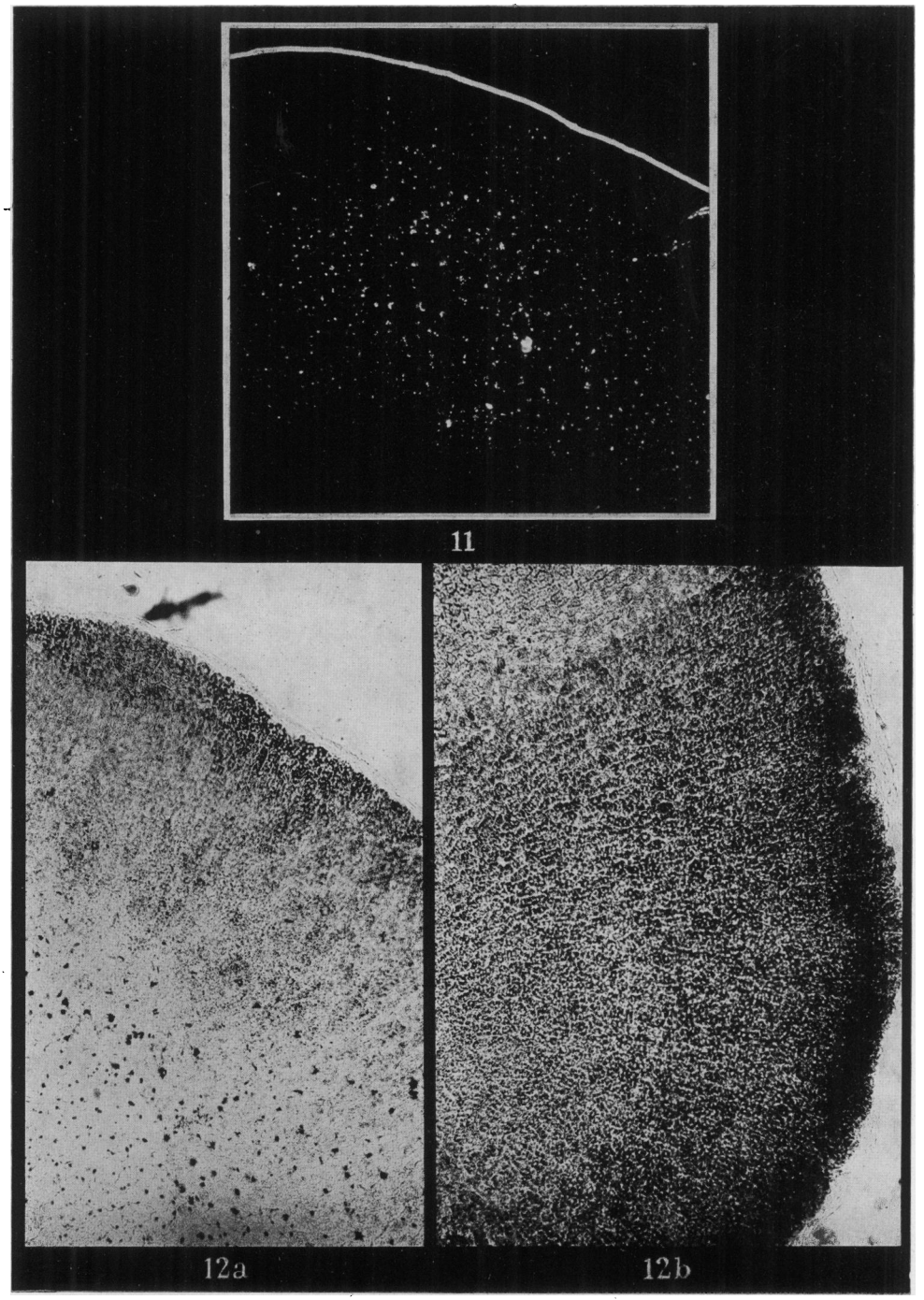

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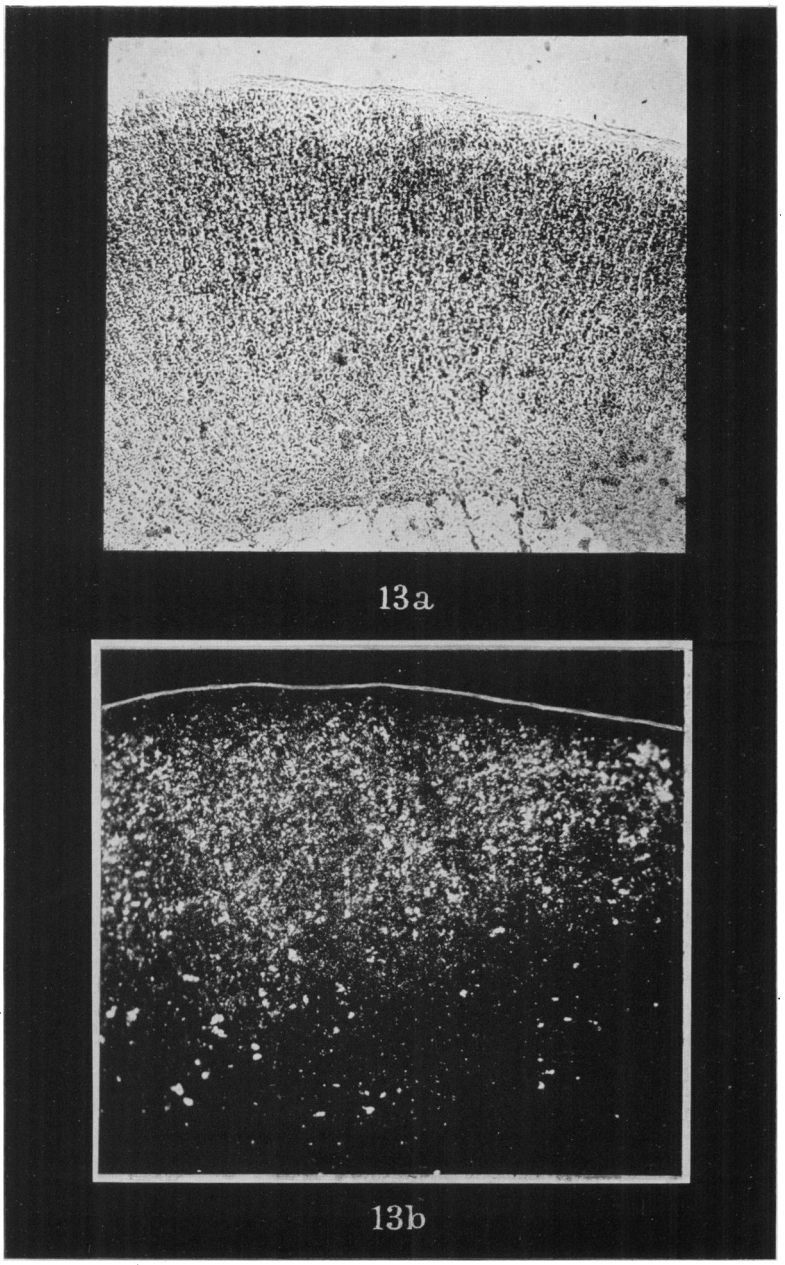

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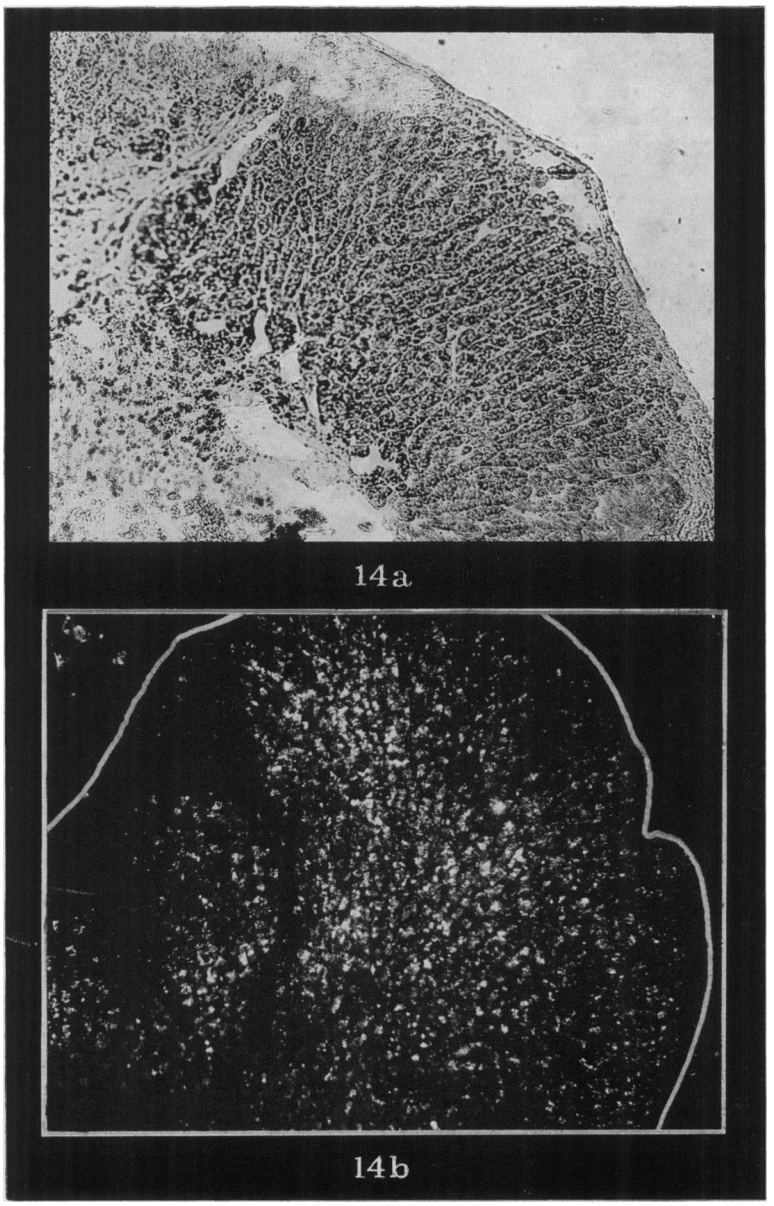

14a

14b

When the adrenal cortex is examined with polarized light droplets containing ketosteroid exhibit birefringence, which may be increased by treating the glands with digitonin to form insoluble anisotropic esters with cholesterol and other β-steroids. Large birefringent particles are associated with storage, whilst small ones are thought to indicate active secretion (Greep and Deane, 1949*a*). In ATP-treated rats both the number and size of the particles was greatly reduced, reaching a minimum 7 hours after the injection (Fig. 10). The decrease was comparable to that seen after the subcutaneous injection of 3 mg. ACTH (Fig. 11). Both with ACTH and ATP the loss of birefringent material was from the cells of the zona fasciculata. The apparent changes in the zona glomerulosa seen in the photomicrographs are artefacts due to the difficulties of focusing, etc.; the birefringence of that zone was, in fact, unaltered. Recovery of the birefringent material occurred within 24 hours when, in fact, the zona fasciculata of the ATP-treated rat usually showed more birefringence than the control (Fig. 10).

These effects of ATP on the histochemistry of the adrenal cortex were seen in both male and female rats, and both in fed animals and those which had been fasted overnight.

Repeated exposure to stress usually leads to adrenal hypertrophy in the first instance. Three rats given 95 mg. MgATP per 100 g. body weight on alternate days for 3 doses were killed 36 hours after the last injection, and their adrenals compared with those from 3 rats similarly treated with equivalent amounts of D-ribose. Although the adrenals from the ATP-treated rats were not significantly heavier than normal they were yellower in colour. The zonae fasciculata and reticularis contained much more sudanophilic lipid than the controls (Fig. 12), and in the zona fasciculata there was more ketone and more birefringent material, chiefly in the form of large masses.

Effect of fatal doses of ATP.

When fatal doses of ATP were given intraperitoneally and the adrenals examined at death, or when the animal was moribund, the histochemical changes described above were exaggerated. Under these conditions the zona fasciculata was practically denuded of sudanophilic, ketone and birefringent material, whilst the zona glomerulosa appeared normal.

Effect of fatal limb ischaemia.

Fatal ischaemic shock was produced by occluding the circulation to both hind limbs for 5 hours. There was little change in the histochemistry of the adrenal cortex during the period of ischaemia, but 7 hours after release of the occlusion the gland presented a similar picture to that seen at the same time after a non-fatal dose of ATP. As before, the changes were confined to the zonae fasciculata and reticularis, but they did not occur as rapidly as after a fatal dose of ATP, nor was the reduction in ascorbic acid so great.

Effect of ATP after hypophysectomy.

To determine the role of the hypophysis in the adrenal response to ATP it was given, in doses within the non-fatal range for normal animals, to 6 rats 3 days after complete hypophysectomy, i.e. before adrenal atrophy had occurred and

whilst the gland appeared superficially normal. In these animals the zona fasciculata retained its histochemical properties after ATP administration, and the marked discharge of ketosteroid material was not observed (Fig. 13). Rats in which the skull was opened but the hypophysis not removed behaved normally after the injection of ATP.

Effect of ATP after adrenal medullectomy.

The effect on the adrenal cortex of normally non-fatal doses of ATP was studied in 15 rats 21 days after removal of the adrenal medullae and in 5 rats 47 days after this operation. ATP appeared to have little effect on the adrenal cortex of these animals (Fig. 14). Even in rats dying after such a dose of ATP the ascorbic acid content, sudanophilia and birefringence of the zona fasciculata appeared normal. This difference between the normal and medullectomized animal is interesting, since Greep and Deane (1949*b*) have shown that the regenerated gland after enucleation has an apparently normal secretory function, and we have found that it responds in the normal way to the subcutaneous injection of ACTH.

Effect of nucleotide derivatives.

To study the mechanism of the ATP effect some of its derivatives were also tested. Compounds were compared with a non-lethal dose of ATP by injecting them intraperitoneally in equimolecular amounts. Given in this way A5MP caused some discharge of sudanophilic and birefringent material from the zona fasciculata, but was much less active then ATP. Adenosine did not alter the appearance of the glands. As already described, D-ribose was used for control purposes, and had no effect on the glands when compared with equivalent volumes of 0·9 per cent NaCl.

Effect of sodium pyrophosphate.

The effect of $Na_4P_2O_7$ (0·1M) in amounts equivalent to non-fatal doses of ATP, on the basis of pyrophosphate content, was observed in 10 rats. Unlike previous series (Bielschowsky and Green, 1944; Green and Stoner, 1950*a*) the strain of rats used in these experiments was more susceptible to $Na_4P_2O_7$ than ATP, so that this dose was either fatal or produced a more severe state of shock than equivalent amounts of ATP. Despite this $Na_4P_2O_7$ did not cause as much histochemical change in the adrenal cortex as ATP. Four hours after the injection the sudanophilia and birefringence of the zona fasciculata appeared normal in 3 of 4 rats. In rats dying after $Na_4P_2O_7$ the ascorbic acid, sudanophilia, birefringence and ketones of this zone were reduced, but less strikingly than in rats examined 7 hours after a non-lethal dose of ATP.

DISCUSSION.

If we accept the various histochemical tests used as giving a fair picture of the functional activity of the adrenal cortex, our results show that ATP shock is accompanied by secretion from the zona fasciculata, and that repeated administration of ATP leads to increased ketosteroid storage in that zone. The effect of ATP on the adrenal cortex, therefore, closely resembles that of other stress-inducing agents and of ACTH. Of the adenyl compounds tested ATP was the

most active, activity being directly related to the shock-inducing potency of the compound. In this connection the results obtained with $Na_4P_2O_7$ are interesting, for whilst the degree of shock was greater than that produced by equivalent amounts of ATP, the adrenal cortical changes were very much less. In earlier work (Bielschowsky and Green, 1944 ; Green and Stoner, 1950*a*) we have tended to consider the shock-inducing actions of ATP and $Na_4P_2O_7$ as being closely related, and to draw the conclusion that this action of ATP is related to its phosphate groups. Whilst other evidence supports this conclusion (Green and Stoner, 1950*a*), the results described above and others obtained recently (Stoner, Green and Threlfall, 1950) show that this similarity is not as great as originally thought. Possible explanations for the smaller effect of $Na_4P_2O_7$ on the cortex such as the inhibition of local enzymic mechanisms associated with secretion have not yet been explored.

The action of ATP on the adrenal cortex of the intact rat is not a direct one, but depends upon the secretions of two other endocrine glands. Although in experiments on the perfused gland Vogt (1949) found that the addition of ATP to the perfusion fluid increased the output of cortical hormone, she considered that this was more probably due to the restoration of normal functioning conditions than to active stimulation. Whilst our methods cannot completely exclude any direct action on the gland, the results provide no evidence for it, and that is certainly not the main pathway of the effect in the intact animal. The experiments on the hypophysectomized and medullectomized rats suggest the following series of events. The injection of ATP first causes the secretion of adrenaline, which in turn leads to the liberation of ACTH from the anterior lobe of the hypophysis, and hence to the changes in the adrenal cortex. Our studies on carbohydrate metabolism in ATP shock (Stoner *et al.*, 1950) provide additional evidence that adrenaline is released in this condition and in amounts sufficient to cause a striking hyperglycaemia, i.e. in amounts of the order required for stimulation of the hypophysis (Ronzoni and Reichlin, 1950). The activity of the other adenyl compounds is in the same order as their hyperglycaemic action. Although it is agreed that both adrenaline and nor-adrenaline will discharge hormones from the adrenal cortex (Vogt, 1944 ; Long and Fry, 1945 ; Long, 1947 ; Naysmyth, 1950), the mechanism of the effect is disputed. Long and his co-workers tend to the view that adrenaline acts through the hypophysis (Long, 1949 ; McDermott, Fry, Brobeck and Long, 1950), whilst Vogt considers that adrenaline can act directly on the adrenal cortex, although she admits that adrenal hypertrophy following repeated doses of adrenaline is dependent upon the hypophysis (Vogt, 1945). Again, it is questionable if the methods we have used are sufficiently sensitive to determine whether or not the adrenaline liberated under these conditions can act directly on the adrenal cortex. Our results show, however, that, as envisaged by Long, the main pathway lies through the hypophysis.

These results may throw some light on the controversy which has arisen over the mechanism of ACTH liberation under conditions of stress. According to Long (1947, 1949) adrenaline forms the link between the stress-inducing agent and the hypophyseal-adrenal cortical response. Sayers and Sayers (1947, 1948), on the other hand, claim that a state of stress in the body leads to greater utilization of cortical hormone, and that the increased demand for hormone leads to the liberation of ACTH and stimulation of the adrenal cortex. Whilst some

mechanism of this sort probably exists (Greep and Deane, 1949*b*), it does not seem to account for the response in stress. Since the increased demand for cortical hormone in ATP shock is presumably as great in the medullectomized rat as in the normal rat, the lack of histochemical response and the greater sensitivity of the former is strong evidence in favour of Long's theory that adrenaline is an essential link in the chain.

Whilst the relationships between the endocrine glands involved in the response to stress become clearer, the gap between the stress-inducing agent and the commencement of that response remains unbridged. Since evidence exists that the adenine nucleotides are concerned in the general bodily reaction to injury (Green and Stoner, 1950*a*, *b*) our present results suggest possible bridges for that gap, but until the extent of their involvement is accurately defined it would be unwise to proceed further with such an hypothesis.

Leaving these considerations aside there is no doubt that, as in other forms of shock (Swingle and Parkins, 1935), the functional state of the adrenal gland is an important factor in determining the outcome of ATP shock. Anything which interferes with the normal discharge of cortical hormones, such as removal of the glands or their inactivation by medullectomy or hypophysectomy, greatly increases the animal's sensitivity to ATP. The relatively greater size of the adrenal in the young animal (Korenchevsky, 1942 and *v.s.*) may partly account for their greater resistance to the shock-inducing action of ATP, especially since the effect of age on sensitivity was not seen after adrenalectomy.

The limitation of the histochemical response to the zona fasciculata suggests that in ATP shock, as in shock from tissue injury, the protective action of the adrenal cortex is exerted through its gluco-corticoid hormones, and that it is their removal which sensitizes the adrenalectomized animal to this stress. A further possibility must, however, be considered, especially in view of the poor response to replacement therapy with either type of hormone. Conway and Hingerty (1946) have shown that in adrenalectomized rats, not maintained on NaCl, and in fairly advanced stages of deficiency, the Mg content of the plasma and muscle is raised. Since Mg^{++} administration greatly increases the toxic effects of ATP and its derivatives (Green and Stoner, 1944, 1950*a*), we determined the plasma Mg content in adrenalectomized rats under our conditions i.e., maintained on NaCl, deprived of food overnight, and apparently in good health, and found it to be within the normal range. Consequently this mechanism would not seem to be concerned in our experiments. The explanation of the poor response to replacement therapy probably lies in the differing potencies of the hormone preparations used. Desoxycorticosterone acetate is a pure substance with marked physiological activity so that its slight effect when given in large doses is hardly striking evidence in favour of the involvement of the mineralo-corticoids in the response, especially in view of its possible conversion to glycogenic material in the body (Hayano, Dorfman and Prins, 1949). In comparison the gluco-corticoid potency of a whole gland extract is low, which increases the significance of its similar effect to desoxycorticosterone acetate in the adrenalectomized animal and its therapeutic effect in the normal one. A valid comparison would be between desoxycorticosterone and cortisone, but supplies of the latter were not available to us.

The mechanism of the protective action of the gluco-corticoids in ATP shock is not clear, nor indeed has a satisfactory explanation been advanced in any form

of shock. Work on this problem is proceeding, and the results will be embodied in a further paper.

SUMMARY.

The role of the adrenal cortex in nucleotide shock has been studied in the rat. It was found that ATP acts like other shock-inducing agents in causing the discharge of hormones from the zona fasciculata of the adrenal cortex as judged by histochemical tests. Derivatives of ATP have a similar effect in proportion to their shock-inducing potency, but $Na_4P_2O_7$ is not nearly as effective as ATP. The effect is not a direct one on the adrenal cortex, but is mediated through the adrenal medulla and hypophysis. The prevention of the response by adrenalectomy, adrenal medullectomy or hypophysectomy increases the animal's sensitivity to ATP. The protective action of the adrenal cortex is thought to be due to its gluco-corticoid hormones. The results are discussed in relation to current theories on the mechanism of the adrenal response to body injury.

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