J. Physiol. (1957) 135, 536–549

# A CYTOCHEMICAL STUDY OF THE ADRENAL CORTEX REGENERATING AFTER ENUCLEATION

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(*Received* 8 *August* 1956)

In recent years detailed studies of the chemical composition of organs undergoing hypertrophy have been published. The liver in hypertrophy after partial hepatectomy has been the organ of choice for these studies, the most comprehensive being those of Price & Laird (1950) and Tsuboi, Yokoyama, Stowell & Wilson (1954). Some investigations have also been made on the kidney in compensatory hypertrophy after unilateral nephrectomy (Mandel, Mandel & Jacob, 1950; Vegni, 1953; Dianzani & Biaggini, 1954). In this case the hypertrophy is essentially localized to the proximal convolutions which comprise a relatively small proportion of the total mass of the kidney (Oliver, 1944–5).

In the present paper the chemical composition has been studied of the adrenal cortex during regeneration after enucleation (Hartman, Brownell & Knouff, 1947). This process can be defined as true regeneration, since it complies with many of the criteria expounded by Cameron (1952). In particular, adrenal regeneration differs from liver hypertrophy after partial hepatectomy because initially the regenerating tissue shows signs of lack of differentiation as evidenced by the absence of normal zones in the cortex during the first month.

One of the main points of this investigation was to attempt to correlate chemical changes occurring during the regeneration with the gradual restoration in the functional abilities of the enucleated gland, this being a continuation of previous work carried out in this Department.

## METHODS

*Animal procedure.* Young virgin female rats of the Italico strain were used, bred in this Department's colony and weighing between 90 and 192 g at the time of death. They were kept on a standard balanced diet (Randoin & Causeret, 1947) supplemented by green vegetables. Enucleation of both adrenals was performed according to Hartman *et al.* (1947), in a single stage by the dorsal approach under light ether anaesthesia. Enucleated animals were not given NaCl in the drinking water.

*Preparation of adrenal fractions.* Groups of rats were killed by stunning and exsanguination at intervals varying from 15 to 90 days after the operation. It was considered that adrenals regenerating for periods shorter than 15 days were not suitable for this study on account of the presence of variable amounts of blood coagulum, necrotic tissue, macrophages and fibroblasts, which could obscure the interpretation of the chemical data (Greep & Deane, 1949; Pellegrino, 1951). The adrenals were quickly dissected, freed of connective tissue and stored at 0° C until all the glands had been collected. Pooling a relatively large number of adrenals (from 24 to 40) was not only necessary in order to secure enough material for the analyses, but also smoothed out individual variations in the extent of regeneration. The pooled glands were weighed and dispersed in a Potter & Elvehjem (1936) homogenizer fitted with a Perspex pestle and surrounded by ice in 9 parts of ice-cold 0·88 M sucrose containing 0·002 M versene (ethylenediaminetetraacetic acid) adjusted to pH 7·2. The time (2·5 min) and conditions of homogenization were carefully controlled to ensure they could be reproduced. Hypertonic sucrose was chosen, because clumps of nuclei were very rarely observed with this medium in contrast to isotonic sucrose.

Regenerating nuclei might be more fragile than normal and more liable to break during the homogenization. This possibility was tested as follows. The nuclear fractions of dispersions of normal and regenerating tissue were separated according to Schneider (1949) in a refrigerated centrifuge (Eispirouette, Phywe). Deoxyribonucleic acid (DNA) was determined separately on the nuclei and the supernatant using the procedure described below. The difference between normal and regenerating tissue was negligible. In all cases at least 92% of the total DNA content of the dispersion was recovered in the nuclear fraction, indicating that our results for nuclear counts and chemical composition of the average cell are approximately correct. The dispersions were kept at 0° C until used for various determinations.

*Nuclear counts.* Suitable aliquots were taken in duplicate from the 1-in-10 tissue dispersion and were diluted a further 1-in-10 or 1-in-20 with a solution containing 0·01% (w/v) gentian violet in 3% (v/v) aqueous acetic acid (Laird, 1953). Nuclei are clearly differentiated by this staining technique. The suspension was shaken thoroughly and the nuclei were counted in a Bürker haematocytometer chamber by two observers within 2 hr after the preparation of the dispersion. At least 1000 nuclei were counted by each person and counts were repeated, if necessary, until the results did not differ by more than 5%.

### *Chemical measurements*

*Total nitrogen* was determined in duplicate or triplicate, by the micro-Kjeldahl method. The digestion was carried out in the presence of potassium persulphate, and ammonia distilled in the Markham (1942) apparatus was collected in 0·0143 N-$H_2SO_4$ for back titration with 0·0143 N-NaOH using Tashiro's indicator.

*Protein nitrogen.* Suitable aliquots of the homogenate (in duplicate or triplicate) were added to 5 ml. of 5% (w/v) trichloroacetic acid (TCA) and after centrifugation the supernatant was carefully decanted. The sediment was resuspended in 3 ml. of 5% (w/v) TCA, centrifuged and after discarding the supernatant dissolved in warm 2 N-NaOH (0·5 ml.) and analysed for N as described above.

*Separation of phosphates, lipids and nucleic acids.* Duplicate aliquots of the homogenate were fractionated according to Schneider (1945) into: (i) cold TCA soluble fraction; (ii) hot ethanol-ether (3:1) soluble fraction; (iii) hot (90° C) TCA soluble fraction, and (iv) residual fraction.

*Phosphorus determinations* were carried out in duplicate on aliquots of fractions (i), (ii) and (iv), previously digested with 1 ml. 60% (v/v) perchloric acid (Ciba), using the method of Lowry, Roberts, Leiner, Wu & Farr (1954) adapted for the use of larger samples. Optical densities were read at 820 mμ in a Beckman Model DU spectrophotometer and compared with standards.

*Estimation of cholesterol.* Free and total cholesterol was determined on aliquots of fraction (ii) according to Popják (1943), using redistilled solvents throughout. The colorimetric assay of cholesterol was performed as described by Sperry & Brand (1943), always running appropriate reagent blanks and standards.

*Fatty acids.* After precipitation of total cholesterol with digitonin and centrifugation, the supernatant fluid was carefully decanted, treated as described by Popják (1943) and fatty acids determined colorimetrically according to Bragdon (1951). The results are expressed in equivalents of stearic acid (Merck, Darmstadt) which was used as a standard.

*Nucleic acids.* Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were determined in the hot TCA soluble fraction (iii) using respectively the diphenylamine method of Dische (1930) and the orcinol method of Mejbaum (1939). Preparations of DNA (Na salt) from herring sperm (Light) and of RNA from yeast (Bayer) were used as standards. These preparations were used without further purification. The atomic extinction coefficients, $\epsilon(P)$, were 7660 for RNA and 5300 for DNA. Sugar reactions were used for the estimation of nucleic acids in preference to *P* determinations according to Schmidt & Thannhauser (1945) since the ribonucleotide fraction from adrenal tissue prepared according to these authors contains appreciable amounts of non-nucleotide phosphate esters (Symington & Davidson, 1956).

*Ascorbic acid.* Immediately after the preparation of the homogenate, 0·1 ml. was added to 5 ml. of 4% (w/v) TCA and the mixture was kept frozen at −18° C until the ascorbic acid was determined according to Roe & Kuether (1943).

### *Enzyme assays*

These were performed within 12 hr of preparing the tissue dispersions, with the exception of cytochrome oxidase, which was tested within 1 hr.

*Estimation of cytochrome oxidase.* This was carried out by the spectrophotometric method of Cooperstein & Lazarow (1951) in a Beckman Model DU spectrophotometer using 1 cm cells. The reaction was started by adding 0·1 ml. of a suitable dilution of the homogenate in 0·03 M phosphate buffer, pH 7·4, to 3 ml. of $1{\cdot}7 \times 10^{-5}$ M cytochrome *c* (Sigma Chemical Co.), in the same buffer, which had previously been reduced with sodium hydrosulphite. Optical densities at 550 mμ were read every 30 sec for 3 min. The blank was then read after adding a small amount of solid potassium ferricyanide to the reaction mixture. The assays were always carried out, at least in duplicate, at two enzyme levels. The oxidation of cytochrome *c* behaved as a first-order reaction with respect to the logarithm of the concentration of reduced cytochrome *c*. The results of the assay are expressed in units, one unit being defined as the activity of the enzyme causing the logarithm of the concentration of reduced cytochrome *c* to decrease by 1/min/10 ml. of incubation mixture containing 100 mg of tissue (wet weight). Since the cell compartment of the spectrophotometer was not thermostatically controlled, the temperature in the cell at the end of each run was recorded and only the results obtained at 19° ± 1° C used.

*Acid and alkaline phosphatase assay.* The activity of these enzymes was measured at 38° C in the presence of 0·005 M sodium di-phenylphosphate (British Drug Houses, Ltd.) as substrate, according to the procedure described by Mathies, Goodman & Palm (1952). The pH of the assay mixtures were 5·6 ± 0·1 and 10·10 ± 0·05 respectively, for the acid and alkaline phosphatase determinations. These were determined at two enzyme levels using 1-in-20 dilutions of the original homogenate in distilled water.

*β-Glucuronidase.* This was assayed as described by Pellegrino & Villani (1956) at pH 5·2, using phenolphthalein glucuronide as substrate. A 1-in-20 dilution of the original tissue dispersion in distilled water was used for the estimation, thus obviating the inhibitory effect of higher sucrose concentrations (Gianetto & deDuve, 1955).

*Presentation of the data.* The results are expressed in terms of concentration per unit wet weight of the tissue, of amount per cell (on the basis of the nuclear counts) and as total amount or total activity of both adrenals/100 g body weight as a percentage of normal (as an expression of the rate of restoration).

RESULTS

*Rate of adrenal regeneration.* Fig. 1 shows that adrenal regeneration in terms of weight of the tissue is slow and the regenerated tissue, even 3 months after the operation, is only 62 % as heavy as the normal gland. However, the weight of the latter includes the medulla which is absent from the regenerating gland, but according to Donaldson (1919) and Jackson (1919) this accounts for less than 10% of the normal weight of the organ. Thus it is evident than even 90 days after the operation the normal weight of the cortex is not restored.

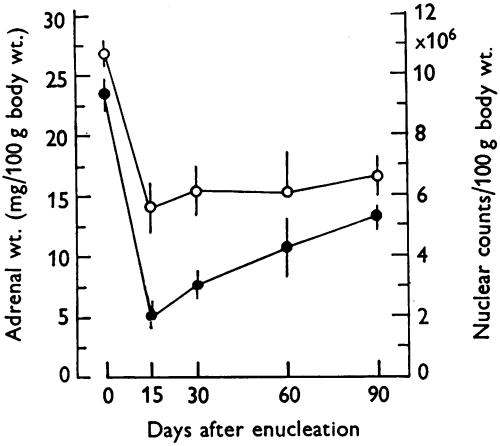


Fig. 1

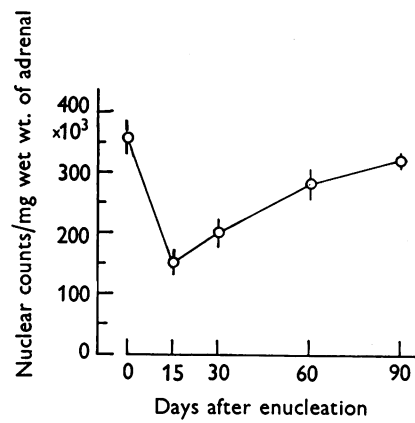


Fig. 2

Fig. 1. Weights and nuclear counts of adrenals during regeneration after enucleation. Results for the two adrenals are expressed in terms of body wt. and represent the means of three or four groups of adrenals (see Methods): ○, adrenal weight; ●, nuclear counts; vertical lines represent 1 or 2 × the S.E.

Fig. 2. Nuclear counts expressed in terms of wet wt. of adrenals. The vertical lines represent 2 × S.E.

Concerning the number of cells in the regenerating tissue, these fell to a very low level (22 % of the normal) 15 days after the operation and then increased gradually to 56 % of the normal after 3 months. The marked variations observed during the course of regeneration in the number of nuclei per mg of tissue (Fig. 2) could indicate considerable modifications in the weight of the average cell. This will be dealt with more fully in the discussion.

*The total and protein nitrogen* contents of the regenerating adrenals run parallel throughout the whole of the experimental period (see Fig. 3). These contents remain approximately constant when expressed on a wet-weight basis, whereas they are both greatly increased on a cell basis, reaching a maximum at 15 days, followed by a gradual decrease towards the normal values.

Even 3 months after the operation, however, both total and protein nitrogen contents are considerably lower than normal.

*Deoxyribonucleic acid.* The average DNA content of the nucleus rose from 11·6 to $31 \times 10^{-9}$ mg during the first 15 days of regeneration. A slow and progressive decrease followed, but even after 3 months the nuclear DNA content was still higher than normal (see Fig. 4). The DNA content per cell thus follows the same pattern of change during regeneration as do the total nitrogen and protein contents (see Figs. 3 and 4).

The concentration of DNA does not vary greatly during the regeneration period, indicating that the synthesis of this nuclear constituent parallels that of other cell constituents. The restoration of the total DNA content of the

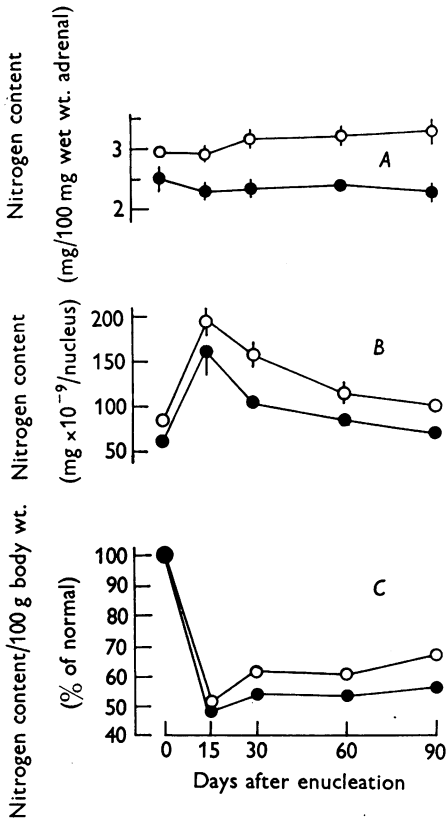


Fig. 3

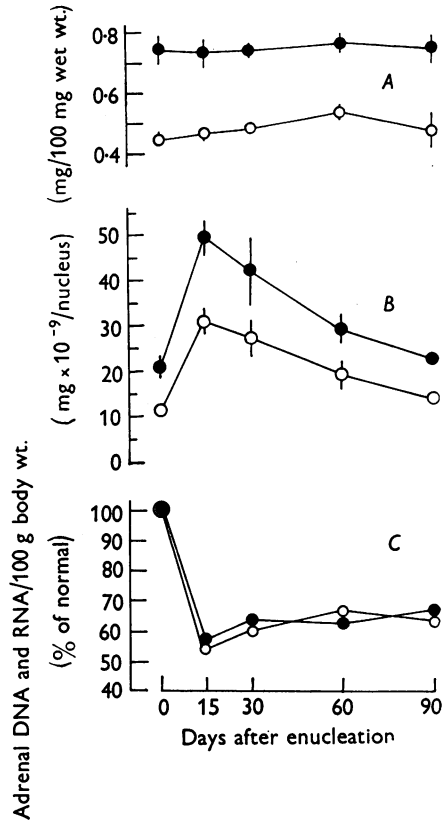


Fig. 4

Fig. 3. Nitrogen content of regenerating adrenals. ○, total N; ●, protein N; results are expressed in terms of wet wt. adrenals (*A*); per nucleus (*B*); and as a percentage of the N content of normal adrenals per 100 g body wt. (*C*). The vertical lines represent 1 or 2 × S.E.

Fig. 4. DNA (○) and RNA (●) contents of regenerating adrenals. Results are expressed in terms of wet wt. adrenals (*A*); per nucleus (*B*); and as a percentage of the DNA or RNA content of normal adrenals per 100 g body wt. (*C*); the vertical lines represent 2 × S.E.

gland is also incomplete, and after 3 months has only reached 65% of the normal.

*The ribonucleic acid* concentration in the regenerating adrenal remains practically constant. The content per cell, however, increases markedly with a maximum, as for other cell constituents, at 15 days, when it is approximately 2·5 times the normal RNA content. At the end of the experimental period, the total RNA content of the regenerating gland is only 67% of the normal.

*Cholesterol.* Results for the free and ester cholesterol contents of the regenerating glands are presented in Fig. 5. Free cholesterol, which constitutes a minor portion of the total adrenal cholesterol, decreased during the first month on a wet-weight basis, and then rose gradually to approach the normal value. The content per cell, however, was increased 15 days after the operation, but thereafter decreased rapidly to reach a normal level after 2–3 months. The rate of restoration was extremely low in the first month and incomplete (58% of normal) after 3 months.

Ester cholesterol behaved differently. Its concentration decreased rapidly at first and then rose to a maximum at 1 month and subsequently slowly declined. The content per cell was practically constant during the first month and then decreased gradually. Its rate of restoration was low and did not exceed 40% of the normal amount.

*Total fatty acids.* The behaviour of the total fatty acids in the regenerating gland is different from that of cholesterol. The concentration in the tissue fell after the operation to a minimal value at 15 days and then rose until it was finally higher than that of the control group. The content per cell, however, was always greater than normal. Following the initial post-operational fall, the total content per 100 g body weight was very rapidly restored, and at the end of the experimental period was 85% of that of the control glands.

*Phosphorus fractions.* Results for the acid soluble, lipid and residual (protein) P contents of the regenerating glands are shown in Fig. 6. The nucleic acids contents have been presented in a previous section. The acid-soluble P content of the regenerating gland, on whatever basis it was expressed, was always higher than the control. These increases above the normal were particularly marked 15 days after the operation when expressed on a weight or cell basis. In contrast to these results, both lipid P and protein P decreased in concentration during the first stages of regeneration; this was followed, initially at least, by an increase. When expressed on a cell basis, both constituents were markedly increased at the beginning of regeneration, but fell towards normal later. The total lipid P and protein P contents of the regenerating glands were not restored to normal by the end of the experimental period.

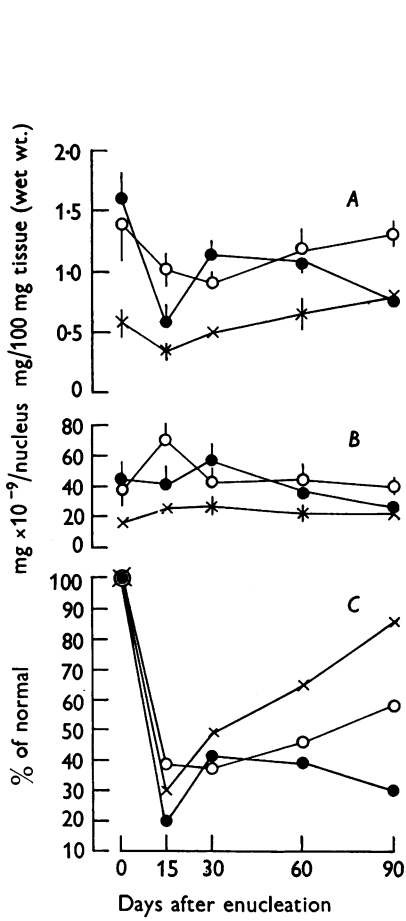


Fig. 5

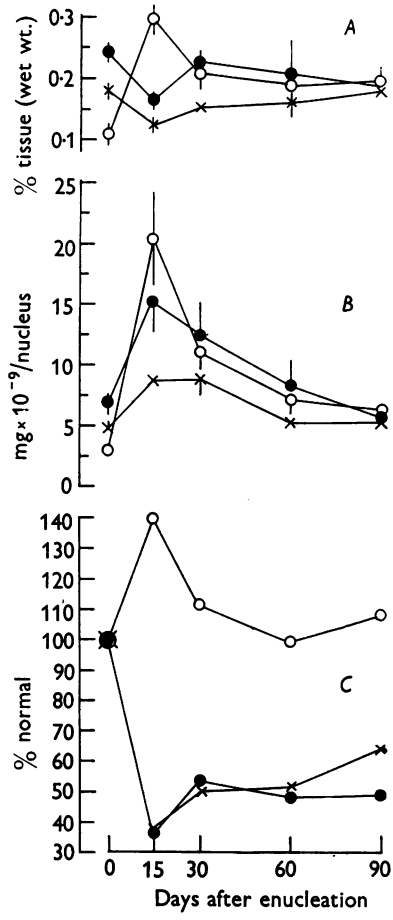


Fig. 6

Fig. 5. Free (○) and ester (●) cholesterol and total fatty acids (×) contents of regenerating adrenals. Results are expressed in terms of wet wt. adrenals (*A*); per nucleus (*B*); and as a percentage of the free or ester cholesterol or total fatty acid contents of normal adrenals per 100 g body wt. (*C*). For convenience, the ordinates for free cholesterol in graphs *A* and *B* have been multiplied by 5, and those for total fatty acids divided by 10. The vertical lines represent 1 or 2 × S.E.

Fig. 6. Phosphorus fractions of regenerating adrenals. ○, acid-soluble P; ●, lipid P; ×, residual P. Results are expressed in terms of wet wt. adrenals (*A*); per nucleus (*B*); and as a percentage of the acid soluble P, lipid P or residual P content of normal adrenals/100 g body wt. (*C*). For convenience, the ordinates for residual P in graphs *A* and *B* have been multiplied by 10. The vertical lines represent 1 or 2 × S.E.

*Ascorbic acid.* Changes in the ascorbic acid content of the regenerating adrenal glands are shown in Fig. 7. The concentration of ascorbic acid was considerably reduced 2 weeks after enucleation, in agreement with previous results (Pellegrino & Montella, 1954). A moderate increase was observed later but the values were always markedly lower than normal. The content per cell was slightly increased 15 days after the operation, but decreased steadily afterwards, reaching about two-thirds of the normal amount after 3 months. After 2–3 months, the total ascorbic acid content of the regenerating glands was only 40% of the normal.

*Cytochrome oxidase.* The activity of cytochrome oxidase (see Fig. 8) showed no substantial variation, on a wet-weight basis, during the first month of regeneration, but decreased somewhat later.

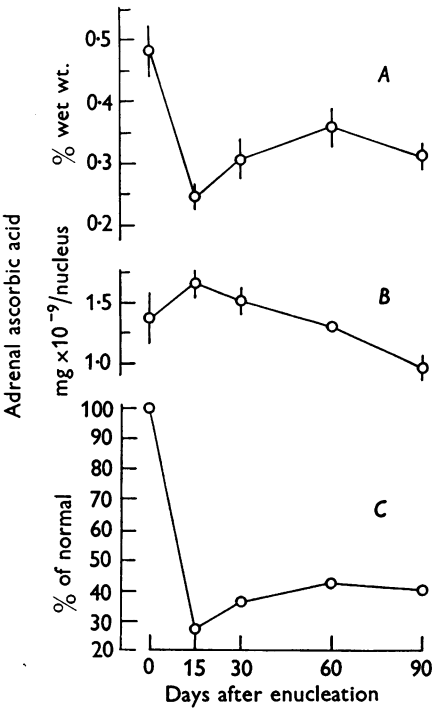


Fig. 7

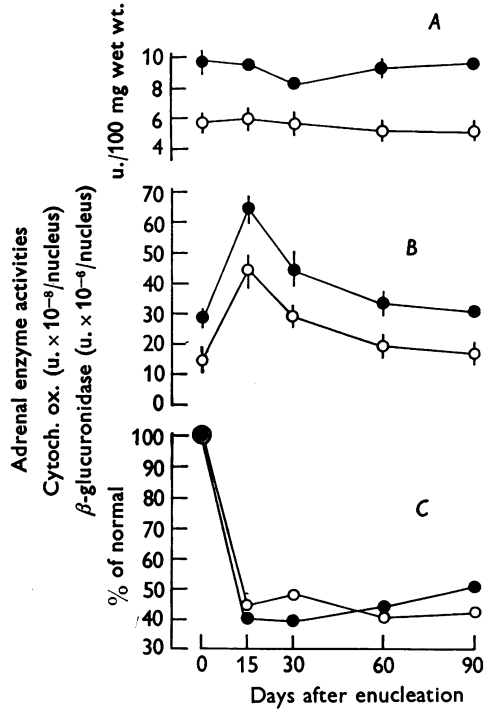


Fig. 8

Fig. 7. Ascorbic acid content of adrenals during regeneration. Results are expressed in terms of wet wt. adrenals (*A*); per nucleus (*B*); and as a percentage of the ascorbic acid content of normal adrenals per 100 g body wt. (*C*). The vertical lines represent 2 × S.E.

Fig. 8. Cytochrome oxidase (○) and β-glucuronidase (●) activities of regenerating adrenals. For convenience, the ordinates for β-glucuronidase in curve *A* have been divided by 100. Results are expressed in terms of wet wt. adrenals (*A*); per nucleus (*B*); and as a percentage of the enzyme activities of normal adrenals per 100 g body wt. (*C*). The vertical lines represent 2 × S.E.

Its activity per cell had increased markedly (almost to three times the normal value) after 15 days, followed by a gradual decline to normal at the end of the experimental period. The total enzyme activity was only restored to about 50% of the normal.

*β-glucuronidase.* The activity of β-glucuronidase was followed during regeneration, since, according to some workers, increases in the activity of this enzyme parallel cell proliferation (Levvy, Kerr & Campbell, 1948; Kerr, Campbell & Levvy, 1949, 1950). In the present investigation, however, β-glucuronidase activity decreased during the first month after the operation, on a wet-weight basis and then returned slowly to normal (Fig. 8). Its activity per cell followed the usual pattern, with a maximal increase at 15 days and a return to normal towards the end of the experimental period.

*Acid and alkaline phosphomonoesterases.* The activity of the two main phosphomonoesterases was investigated at both pH 5·6 ('acid' enzyme) and 10·05 ('alkaline' enzyme). The results are shown in Fig. 9. A decrease in activity of both enzymes on a wet-weight basis occurred during the first month of regeneration, this being followed by a return to normal in the case of alkaline phosphatase. The activity per cell and the rate of restoration followed the pattern of the other enzymes.

## DISCUSSION

The results of the present investigation show that the regenerating adrenal gland, even 3 months after enucleation, is still markedly deficient in weight (which corresponds to 62% of the normal) and in total number of cells (which corresponds to 56% of the normal) even when the absence of the medulla, which fails to regenerate, is allowed for. The restoration of the great majority of the chemical components of the tissue is also deficient, because their concentration in terms of wet weight of the tissue is normal or low. The only component which behaves differently is acid-soluble phosphorus, the absolute amount being normal at the end of the experimental period.

In the course of the regeneration the average adrenal weight, which corresponded to 52% of the normal 15 days after enucleation, rose to 58% after 1 month and in the following 2 months increased only from 58 to 62%. It is therefore apparent that the gland reached an almost stationary condition, from the point of view of weight, after 3 months. Moreover, it is clear that the greatest rate of regeneration occurred between the operation and the 15th day, by which time almost half of the normal adrenal weight had been restored. The presence in the gland of a blood coagulum and of a small amount of necrotic material and scar connective tissue, however, prevents precise determination of the rate of growth during this period. The present study was therefore restricted to a later stage of regeneration, extending from 15 days onwards. It corresponds to the last period of weight increase, which follows an early stage

of rapid growth, and to a period of cellular readjustment which lasts for some months.

Since the weight of the gland reached an almost stationary stage 3 months after the operation, at a level still notably lower than normal, factors preventing the full restoration of organ weight are probably implicated. The total number of cells (nuclear counts) in the regenerating adrenals increases progressively from 22 % of the normal at 15 days to 56 % after 3 months. This contrast between the marked increase in cell number and the very slow increase

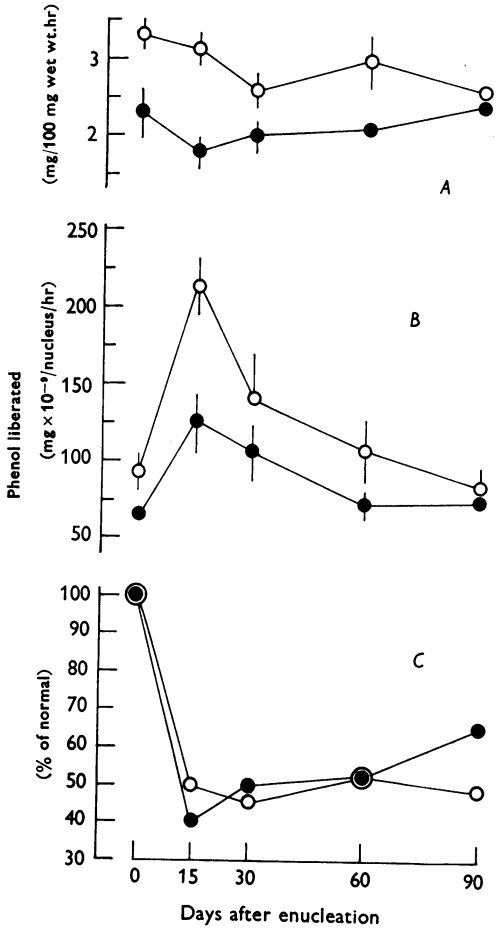


Fig. 9. Acid (○) and alkaline (●) phosphatase activities of regenerating adrenals: results are expressed in terms of wet wt. adrenals (*A*); per nucleus (*B*); and as a percentage of the enzyme activities of normal adrenals per 100 g body wt. (*C*). The vertical lines represent 1 or 2 × S.E.

in the weight of the gland during this period could be ascribed either to the disappearance of extracellular material and its substitution by regenerated cells, or to marked variations in cell size during the course of the regeneration. A relatively constant structural composition of the gland was indicated by histological observations which showed that 15 days after enucleation the mass of connective tissue which had developed in the centre of the gland and between the epithelial regenerating nodules as a consequence of the early healing phenomena was markedly reduced and after 1 month had almost completely disappeared.

It is therefore probably correct to conclude that the lack of correlation between the weight of the gland and the number of cells during the course of regeneration is due chiefly to marked variations in the average cell size. This is maximum after 15 days, when the average regenerating cell apparently weighs more than twice the normal cell. Later, especially after the first month, while the absolute number of cells continues to increase rapidly, the weight of the organ increases very slowly, indicating that the average cell is presumably smaller. This interpretation is supported by data on other cell constituents, in particular total nitrogen, DNA and RNA. It appears therefore that in the course of the second and third month of regeneration some structural rearrangement takes place in which large cells are replaced by smaller ones. As mentioned previously, no data are available on early stages of regeneration.

Our observation, that the weight of the adrenal cell is restored practically to normal when the regeneration of the mass of the tissue is far from being complete, differs from the results of Tsuboi *et al.* (1954) on liver hypertrophy after partial hepatectomy in the mouse. In this case the weight of the average cell was considerably greater than normal at the stage when the organ had recovered its original weight, and also for a considerable period afterwards. The mechanisms by which an organ recuperates its weight seem therefore to be different from those operating in the recovery of the normal weight of the average cell.

The data on total and protein nitrogen substantiate the conclusions drawn from the analyses of wet weight since they show variations in the same direction and approximately of the same magnitude. The average DNA content of the cell during regeneration is high, particularly after 15 days, when it is more than 2·5 times the normal amount. In this connexion the histological observations show that many nuclei are considerably increased in size, this being particularly evident 15 days after enucleation. The present results on the DNA content of the cells are in agreement with those of Price & Laird (1950), Thomson, Heagy, Hutchison & Davidson (1953) and Tsuboi *et al.* (1954) on regenerating liver. In view of these data and of those of Leuchtenberger & Schrader (1952) and Roels (1956) which show that when the functional conditions vary the amount of DNA per nucleus does not correspond to the values found under normal conditions, it is apparent that when a tissue is growing or

undergoing functional variations its DNA content cannot be considered an indication of the number of nuclei, and therefore of cells, present.

As regards the RNA, no variations in concentration were observed in the regenerating tissue and the RNA content per cell altered in parallel with the total nitrogen and DNA.

During the course of adrenal regeneration other cell constituents behaved similarly to proteins and nucleic acids, in respect to concentration, amount per average cell and rate of restoration, indicating that the chemical composition of the tissue remained fairly constant for long periods, irrespective of the fact that the size of the cells varied considerably. Some constituents, however, showed variations which were sometimes pronounced. Among these are free and ester cholesterol and ascorbic acid, which vary with changes in activity of the adrenal cortex. Their contents per cell were slightly increased at the beginning of regeneration but decreased afterwards. On the contrary, fatty acids showed an initial decrease in concentration, followed by a marked increase. Their amount per cell was always greater than normal.

Acid-soluble phosphorus was the only component investigated which did not decrease, in absolute amount, as a result of enucleation. Its concentration and amount per cell were always much greater than normal. An analogous behaviour of acid-soluble P is recorded by Tsuboi, Stowell & Lee (1951) in liver regenerating after treatment with carbon tetrachloride, but not after partial hepatectomy (Tsuboi *et al.* 1954). The results of the present investigation seem to indicate that the increased acid-soluble P might be related to the actual process of regeneration rather than to regressive changes, as suggested by Tsuboi *et al.* (1954).

The activities of the enzymes investigated behaved in a similar manner to the majority of cell constituents, except that acid and alkaline phosphatase and $\beta$-glucuronidase decreased in concentration during the first month of regeneration, an observation which has already been made in other regeneration processes.

The present and previous observations (see for references Brogi, 1956) show that although the regenerating gland is notably deficient in weight, in number of cells and also in many of its chemical constituents, it possesses an almost normal functional activity. Also, the marked differences in the size of the average cell observed during the regeneration are not apparently correlated with variations in the over-all functional activity of the gland.

## SUMMARY

1. The chemical composition of the adrenal cortex regenerating after enucleation has been studied in the period between 15 days and 3 months after the operation.

2. The determinations included total and protein N, DNA and RNA, acid-soluble P, lipid P and residual (protein) P, free and ester cholesterol, total fatty acids, ascorbic acid and the activities of cytochrome oxidase, acid and alkaline phosphatase and β-glucuronidase. The results are expressed on the basis of wet weight, nuclear counts and as a percentage of the total content or activity of normal adrenal glands.

3. The restoration of adrenal weight, cell number and of all the components determined, with the exception of acid-soluble P, was incomplete.

4. The size of the average cell and the content per cell of many constituents, including DNA, were much greater than normal during most of the experimental period, with a maximum at 15 days, followed by a decline to normal values after 3 months.

5. The interpretation of these findings is discussed.

The authors wish to express their thanks to Professor E. Puccinelli for advice and interest in this work, to Miss Franca Dell'Aiuto for skilled technical assistance in the first stages of the work and to Professor P. Niccolini and Dr L. Mazzanti, of the Department of Pharmacology of this University, for kindly allowing the use of their Beckman spectrophotometer. They are also indebted to Mr E. Artigiani for the care of the animals.

## REFERENCES

Bragdon, J. H. (1951). Colorimetric determination of blood lipids. *J. biol. Chem.* **190**, 513–517.

Brogi, M. P. (1956). The effect of regeneration of the adrenal cortex on the water content of the uterus following oestrogenic stimulation. *J. Endocrin.* **14**, 78–81.

Cameron, G. R. (1952). *Pathology of the Cell*, 1st ed. p. 434. Edinburgh: Oliver and Boyd.

Cooperstein, S. J. & Lazarow, A. (1951). A microspectrophotometric method for the determination of cytochrome oxidase. *J. biol. Chem.* **189**, 665–670.

Dianzani, M. U. & Biaggini, G. C. (1954). Alcuni aspetti citochimici dell'ipertrofia renale di compenso. *Arch. Sci. biol., Napoli*, **38**, 582–596.

Dische, Z. (1930). Über einige neue charakteristische Farbreaktionen der Thymonucleinsäure und eine Mikromethode zur Bestimmung derselben in tierischen Organen mit Hilfe dieser Reaktionen. *Mikrochemie*, **8**, 4–32.

Donaldson, J. C. (1919). The relative volumes of the cortex and medulla of the adrenal gland in the albino rat. *Amer. J. Anat.* **25**, 291–298.

Gianetto, R. & deDuve, C. (1955). Tissue fractionation studies. 4. Comparative study of the binding of acid phosphatase, β-glucuronidase and cathepsin by rat-liver particles. *Biochem. J.* **59**, 433–438.

Greep, R. O. & Deane, H. W. (1949). Histological, cytochemical and physiological observations on the regeneration of the rat's adrenal gland following enucleation. *Endocrinology*, **45**, 42–56.

Hartman, F. A., Brownell, K. A. & Knouff, R. A. (1947). Increased fat factor production after adrenal enucleation. *Endocrinology*, **41**, 213–219.

Jackson, C. M. (1919). The postnatal development of the suprarenal gland and the effects of inanition upon its growth and structure in the albino rat. *Amer. J. Anat.* **25**, 221–290.

Kerr, L. M. H., Campbell, J. G. & Levvy, G. A. (1949). β-Glucuronidase as an index of growth in the uterus and other organs. *Biochem. J.* **44**, 487–494.

Kerr, L. M. H., Campbell, J. G. & Levvy, G. A. (1950). Further observations on changes in β-glucuronidase activity in the mouse. *Biochem. J.* **46**, 278–284.

Laird, A. K. (1953). Nuclear changes induced in rat liver cells by thioacetamide. *Arch. Biochem. Biophys.* **46**, 119–127.

Leuchtenberger, C. & Schrader, F. (1952). Variation in the amounts of desoxyribose nucleic acid (DNA) in cells of the same tissue and its correlation with secretory function. *Proc. nat. Acad. Sci., Wash.*, **38**, 99–105.

LEVVY, G. A., KERR, L. M. H. & CAMPBELL, J. G. (1948). β-Glucuronidase and cell proliferation. *Biochem. J.* **42**, 462–468.

LOWRY, O. H., ROBERTS, N. R., LEINER, K. Y., WU, M. L. & FARR, A. L. (1954). The quantitative histochemistry of brain. I. Chemical methods. *J. biol. Chem.* **207**, 1–17.

MANDEL, P., MANDEL, L. & JACOB, M. (1950). Evolution des acides nucléiques au cours de l'hypertrophie rénale compensatrice. *C.R. Acad. Sci., Paris*, **230**, 786–788.

MARKHAM, R. (1942). A steam distillation apparatus suitable for micro-Kjeldahl analysis. *Biochem. J.* **36**, 790–791.

MATHIES, J. C., GOODMAN, E. D. & PALM, L. (1952). Effects of hypophysectomy, growth hormone and thyroxine on phosphatases of tibia, liver, spleen and kidneys of rats. *Amer. J. Physiol.* **168**, 352–365.

MEJBAUM, W. (1939). Über die Bestimmung kleiner Pentosemengen, insbesondere in Derivaten der Adenylsäure. *Hoppe-Seyl. Z.* **258**, 117–120.

OLIVER, J. (1944–5). New directions in renal morphology: a method, its results and its future. *Harvey Lect.* Series 40, 102–155.

PELLEGRINO, C. (1951). Alcuni aspetti istologici ed istochimici del surrene rigenerante dopo enucleazione. *Mem. Soc. tosc. Sci. nat.* **58** B, 5–14.

PELLEGRINO, C. & MONTELLA, A. (1954). Colesterolo ed acido ascorbico nel surrene rigenerante. *Arch. Sci. biol., Napoli*, **38**, 338–348.

PELLEGRINO, C. & VILLANI, G. (1956). β-Glucuronidase in lymphatic tissues. *Biochem. J.* **62**, 235–240.

POPJÁK, G. (1943). Colorimetric determination of total, free and ester cholesterol in tissue extracts. *Biochem. J.* **37**, 468–470.

POTTER, V. R. & ELVEHJEM, C. A. (1936). A modified method for the study of tissue oxidations. *J. biol. Chem.* **114**, 495–504.

PRICE, J. M. & LAIRD, A. K. (1950). A comparison of the intracellular composition of regenerating liver and induced liver tumours. *Cancer Res.* **10**, 650–658.

RANDOIN, L. & CAUSERET, J. (1947). Constitution d'un régime alimentaire équilibré pour les jeunes rats blancs destinés au dosage des vitamines A et D par les méthodes biologiques. *Bull. soc. sci. Hyg. aliment.* **35**, 14–22.

ROE, J. H. & KUETHER, C. A. (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J. biol. Chem.* **147**, 399–407.

ROELS, H. (1956). La teneur en acide désoxyribonucléique du noyau de la cellule thyroidienne du rat blanc dans diverses conditions expérimentales. *Arch. Biol., Paris*, **67**, 211–268.

SCHMIDT, G. & THANNHAUSER, S. J. (1945). A method for the determination of desoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. *J. biol. Chem.* **161**, 83–90.

SCHNEIDER, W. C. (1945). P compounds in animal tissues. I. Extraction and estimation of deoxypentose nucleic acid and of pentose nucleic acid. *J. biol. Chem.* **161**, 293–304.

SCHNEIDER, W. C. (1949). Methods for the isolation of particulate components of the cell. In UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F., *Manometric Techniques and Tissue Metabolism.* Minneapolis: Burgess.

SPERRY, W. M. & BRAND, F. C. (1943). The colorimetric determination of cholesterol. *J. biol. Chem.* **150**, 315–324.

SYMINGTON, T. & DAVIDSON, J. N. (1956). The effect of exogenous ACTH and conditions of stress on the chemical composition of the human adrenal gland. *Scot. med. J.* **1**, 15–31.

THOMSON, R. Y., HEAGY, F. C., HUTCHISON, W. C. & DAVIDSON, J. N. (1953). The deoxyribonucleic acid content of the rat cell nucleus and its use in expressing the results of tissue analyses, with particular reference to the composition of liver tissue. *Biochem. J.* **53**, 460–474.

TSUBOI, K. K., STOWELL, R. E. & LEE, C. S. (1951). Chemical alterations induced in mouse liver following a single feeding of carbon tetrachloride. *Cancer Res.* **11**, 87–93.

TSUBOI, K. K., YOKOYAMA, H. O., STOWELL, R. E. & WILSON, M. E. (1954). The chemical composition of regenerating mouse liver. *Arch. Biochem. Biophys.* **48**, 275–292.

VEGNI, L. (1953). Contenuto e distribuzione degli acidi nucleici nel rene normale ed in quello ipertrofico. *Arch. Sci. biol., Napoli*, **37**, 454–473.