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THE EFFECT OF COLCHICINE ON THE ENZYME CONTENT OF REGENERATING RAT LIVER AND ON THE PRESSOR AMINE CONTENT OF THE ADRENAL

BY

JOYCE HAWKINS AND J. M. WALKER

From the Department of Pharmacology, University of Oxford

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Higgins and Anderson (1931) showed that if the median and left lateral lobes of the liver (amounting to about 65 per cent of the whole organ by weight) are removed from a rat at a single operation, the animal survives, and the remaining lobes increase in size so that the liver has regained its original weight within about 30 days. The present work describes the changes in amine oxidase and DOPA-decarboxylase content of the liver during its regeneration after partial hepatectomy. These enzymes were chosen because of their contrasted properties ; amine oxidase is insoluble and attached to the mitochondria (Cotzias and Dole, 1951), while DOPA-decarboxylase is soluble and is presumably dispersed throughout the cytoplasm of the cell. Since restoration of these enzymes might be affected by abnormal nuclear division, and since Brues and Jackson (1937) have shown that colchicine inhibits mitosis in regenerating liver, we have also studied the effect of colchicine on the enzyme content.

In view of the possibility that DOPA-decarboxylase plays a part in the formation of adrenaline, the pressor amine content of the adrenals of rats treated with colchicine has also been determined.

METHODS

Partial hepatectomy.—Rats of about 200 g. weight were used and the operation was carried out as described by Higgins and Anderson (1931). The animals were fed both before and after the operation on the stock laboratory diet of cubes and water.

Amine oxidase determinations.—Liver tissue was homogenized in 0.067M-sodium phosphate buffer (*p*H 7.4), and the homogenate was dialysed against tap water in order to reduce the oxygen uptake of the enzyme blank. The concentration of the homogenate was brought up to 1 g. fresh weight of tissue in 10 ml. by the addition of more phosphate buffer. Enzymic determinations were made in conical Warburg flasks, the main compartment of which contained 1 ml. homogenate, 0.4 ml. 0.067 M-sodium phosphate buffer and 0.2 ml. 0.1M-semicarbazide. The side-bulb contained 0.4 ml. 0.05M-tyramine hydrochloride or 0.4 ml. water in the blank, while the inner compartment contained 0.3 ml. N-KOH. Determinations were made at 37° C. in a gas-phase of oxygen. Readings were taken at 5-min. intervals, and the rate of oxidation was calculated from the 5–20 min. readings.

DOPA-decarboxylase determinations.—Liver extracts were prepared and determinations carried out by the method described by Sloane Stanley (1949).

Adrenal extracts.—The adrenals were removed, carefully dissected free from connective tissue, and weighed. They were then ground up in phosphate buffer (*p*H 6.5) so as to make an extract containing 10 mg. fresh tissue per ml. This extract was immersed for 90 sec. in boiling water to precipitate the proteins and was then centrifuged. The pressor amine content of the supernatant fluid was estimated by comparing its action on the blood pressure of a spinal cat with that of a standard solution of synthetic *l*-adrenaline.

RESULTS

We first determined the mean percentage weight of liver removed at operation on 12 rats, and found it to be 63 ± 0.73. Assuming that this amount of liver was removed on each occasion the original total weight of the liver could be calculated. The course of restoration of the liver weight was followed by recording the weight of liver removed at partial hepatectomy and that removed on killing at intervals of 1, 2, 3, 7, 12, and 20 days after operation. The results are shown in Fig. 1, where the weight of the regenerating liver is expressed as a percentage of the original total liver weight.

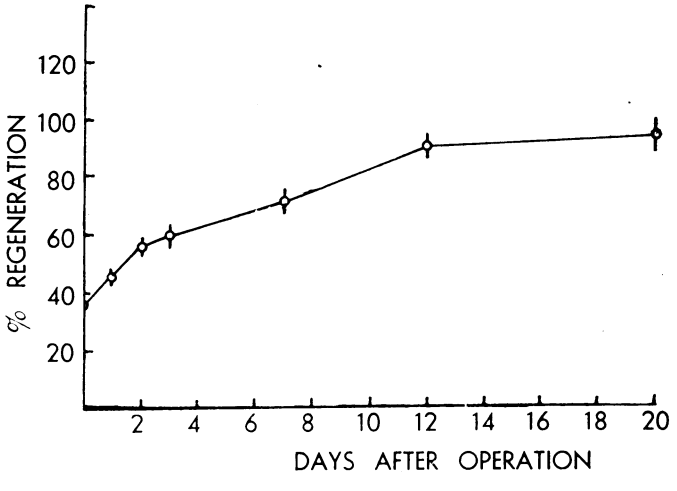

FIG. 1.—Regeneration of liver tissue after partial hepatectomy. Ordinates: wt. of regenerating lobes as percentage of the initial wt. of the liver before operation. Abscissae: days after operation. The points are means of a number of observations ± S.E. of the mean.

Restoration of amine oxidase.—Determinations of amine oxidase activity were made on the liver tissue removed both at operation and at various intervals after. The activity of the regenerating tissue was calculated in each case as a percentage of the activity of the lobes removed at operation, and the results are shown in Fig. 2. In Fig. 2*a* they are expressed as total activity of the liver. It will be seen that there was no change in the total activity for the first two days after operation, and that thereafter there was a gradual restoration of enzyme which roughly followed the course of the growth of liver substance. Fig. 2*b* shows the enzyme activity expressed in terms of unit weight of fresh liver tissue. Here there was a fall in the concentration of the enzyme during the first two days, followed by a rapid rise

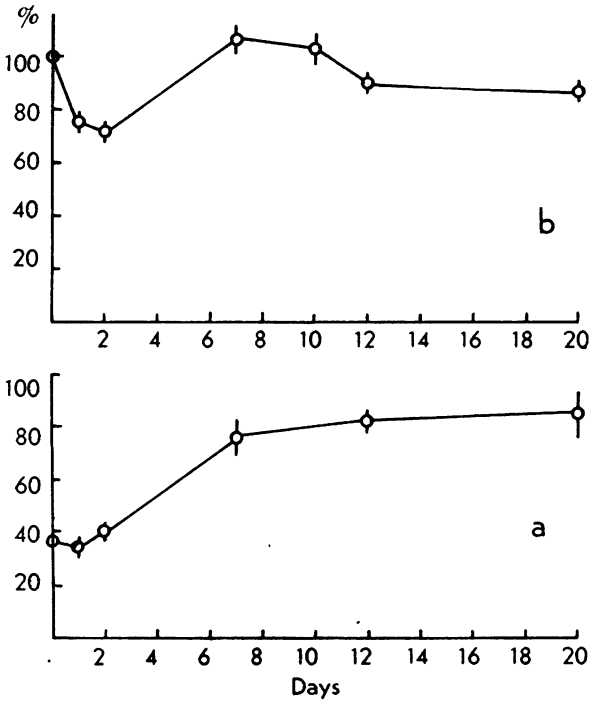

FIG. 2. — Regeneration of amine oxidase after partial hepatectomy. (*a*) qO_2 amine oxidase of regenerating lobes as percentage of the qO_2 of the lobes removed at operation; (*b*) total amine oxidase of regenerating lobes as percentage of that of the whole liver at partial hepatectomy.

until it had reached its original level seven days after operation. Between the seventh and twelfth days there was a fall which was not restored during the subsequent week. Thus three weeks after partial hepatectomy the activity per unit weight was still only about 85 per cent of that before operation. When the enzyme activity was expressed in terms of mg. protein nitrogen, it was found that there was no difference in activity 1, 7, 12, and 20 days after operation, showing that the regeneration of the enzyme protein went parallel with that of the other protein components of the cell.

Restoration of DOPA-decarboxylase.—The activity of DOPA-decarboxylase in regenerating liver is shown in Fig. 3. It will be seen that the restoration of the enzyme was similar to that of amine oxidase and that the total activity was restored to about 80 per cent of its original value in three weeks. As with amine oxidase, there was an initial fall in the concentration of the enzyme during the first day after operation.

The effect of colchicine.—Twenty-four hours after partial hepatectomy there is a marked increase of cell-division. If at this point colchicine is given in a dose of 0.01 to 0.2 mg. per 100 g. body weight, mitosis is interrupted and 12 to 24 hours later numerous abnormal mitotic figures may be seen (Brues, Drury, and Brues, 1936). We therefore gave colchicine (0.1 mg./100 g.) subcutaneously to rats 24 hours after partial hepatectomy. It was found that the rats usually survived this dose of colchicine, but lost their appetite and became lethargic.

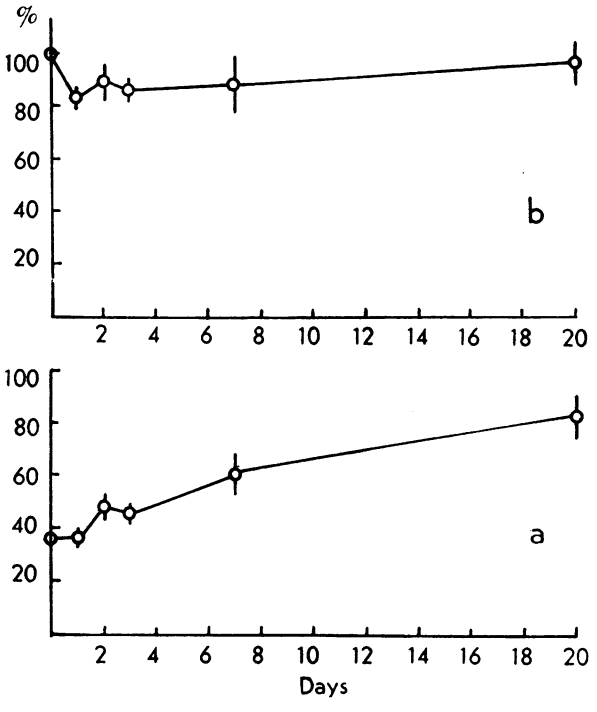

FIG. 3.—Regeneration of DOPA-decarboxylase after partial hepatectomy. Units as in Fig. 2.

(*a*) *Amine oxidase.*—We found that colchicine had no effect on the total amine oxidase content (Fig. 4*a*). When, however, the activity was expressed in terms of unit weight of fresh liver tissue (Fig. 4*b*) it was found that the activity was increased. This result is explained by the effect of colchicine on total liver weight (Fig. 4*c*). It

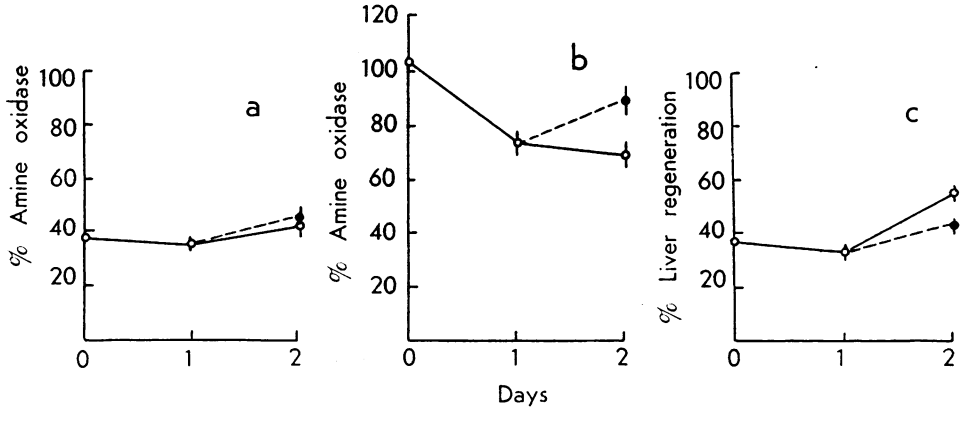

FIG. 4.—The effect of colchicine on the amine oxidase activity of regenerating liver. (*a*) Total enzyme content; (*b*) enzyme activity per unit weight of tissue; (*c*) liver weight. Untreated ——O——; colchicine treated – – –●– – –.

will be seen that in untreated animals there was in the first two days an increase in the weight of the liver without much increase in the total enzyme content. This means that there was a decrease in the activity per unit weight. When colchicine was given, however, there was no increase in the weight of liver during the second day. There was, however, an increase in the total enzyme content, and thus the concentration of the enzyme was greater.

(*b*) *DOPA-decarboxylase.*—The effect of colchicine on the enzymic activity of the liver is shown in Table I. Each result is expressed in two ways. The figure

TABLE I

THE EFFECT OF COLCHICINE ON THE L-DOPA-DECARBOXYLASE ACTIVITY OF THE LIVER IN NORMAL AND PARTIALLY HEPATECTOMIZED RATS

Unoperated rats									Hepatectomized rats			
Controls			Colchicine per 100 g.						Controls		Colchicine per 100 g.	
			0.1 mg.		0.5 mg.		1.0 mg.				0.1 mg.	
	qCO_2	Total activity	qCO_2	Total activity	qCO_2	Total activity	qCO_2	Total activity	qCO_2	Total activity	qCO_2	Total activity
	0.51	2,310	0.43	2,470	0.11	465	0.14	590	0.36	610	0.28	525
	0.78	3,780	0.44	2,300	0.19	955	0.16	670	0.51	965	0.22	325
	0.86	3,150	0.55	3,160	0.35	1,580	0.27	1,070	0.56	945	0.15	257
	0.76	3,550	0.35	1,920			0.12	580	0.50	920	0.32	690
	0.88	4,100	0.59	2,650			0.24	970	0.56	910	0.39	640
	1.35	5,500	0.44	1,960							0.17	295
	0.65	2,950										
Mean	0.83	3,620	0.47	2,410	0.22	1,000	0.19	780	0.50	870	0.25	455
S.E.		±381		±190		±323		±102		±66		±77

on the left is the activity per unit weight of tissue (μl. CO_2 evolved per mg. liver per hr.); that on the right is the total activity of the liver and this figure has been corrected for differences in the body-weights of the rats. Means and standard errors are given at the bottom of the table. It will be seen that in normal rats colchicine in doses of 0.1, 0.5, and 1.0 mg. per 100 g. markedly reduced the enzymic activity, and that the higher the dose the greater was the reduction. In hepatectomized rats 0.1 mg. colchicine per 100 g. also reduced the activity, but no results are recorded for 0.5 and 1.0 mg., since the animals did not survive these amounts.

To compare the effect of colchicine on DOPA-decarboxylase with its effect on amine oxidase, the results of giving 0.1 mg./100 g. of the drug to hepatectomized animals are also shown in Fig. 5. It will be seen that the total enzyme content was reduced by about 50 per cent.

It seemed possible that colchicine might be acting as a specific inhibitor of the enzyme. To test this point the activity of normal rat liver was determined in the presence of colchicine *in vitro*. It was found that the addition of 10 μg. colchicine to 0.75 g. fresh liver had no effect on the enzymic activity.

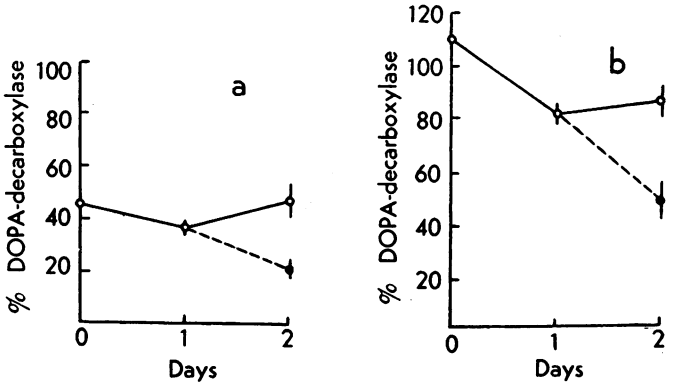

FIG. 5.—The effect of colchicine on the DOPA-decarboxylase activity of regenerating liver. (*a*) Total enzyme content; (*b*) enzyme activity per unit weight of tissue. Untreated——O——; colchicine treated - - -●- - -.

It has been shown that in rats deficient in pyridoxine the DOPA-decarboxylase activity of the liver is very low, and that this can be restored to normal, if the deficiency is not too severe, by the addition *in vitro* of pyridoxal phosphate (Blaschko, Carter, O'Brien, and Sloane Stanley, 1948). In order to see whether the low activity in animals treated with colchicine was due to a deficiency of the coenzyme, synthetic codecarboxylase was added to the liver extract *in vitro*. It was found that in rats partially hepatectomized and treated with colchicine the qCO_2 of the pooled extracts of liver tissue was 0.19. The addition of 10 μg. synthetic codecarboxylase to the pooled extracts *in vitro* resulted in a slight increase in the qCO_2 to 0.29 but did not restore it to the qCO_2 of 0.64 which was found in untreated rats two days after partial hepatectomy. Thus the reduced activity after colchicine was only partly due to loss of coenzyme.

(*c*) *Pressor amines.*—The effect of colchicine on the pressor amine content of the adrenals is shown in Table II. The figures on the right of each column are the pressor amine contents of the glands expressed as μg. adrenaline per 100 g. rat. It will be seen that when doses of 0.1 and 0.5 mg. colchicine per 100 g. were given to normal animals, the pressor amines were not affected. When, however, 1.0 mg. was given to normal rats, or 0.1 mg. to hepatectomized rats, the pressor amine content of the adrenals was much reduced (see also Fig. 6).

DISCUSSION

We have shown that after partial hepatectomy the restoration of amine oxidase and DOPA-decarboxylase proceeded roughly parallel with the restoration of liver weight. There were, however, some discrepancies. First, although during the first two days after operation the total enzymic activity remained almost unchanged (Figs. 2*a* and 3*a*), the liver gained rapidly in weight (Fig. 1), so that the concentration of the enzyme fell (Figs. 2*b* and 3*b*). Apparently the fall in concentration resulted from a " dilution " of the enzymes already present immediately after hepatectomy and no new enzyme was formed until after the second day, i.e., when the cell number began to increase. This explanation is supported, at least for amine oxidase, by our finding that when expressed in terms of mg. protein nitrogen the concentration of enzyme remained constant. The fall in concentration of amine oxidase between the 10th and 20th days (Fig. 2*b*) may be explained in a similar way,

TABLE II

THE EFFECT OF COLCHICINE ON THE PRESSOR AMINE CONTENT OF THE ADRENALS OF NORMAL AND PARTIALLY HEPATECTOMIZED RATS

The results are expressed as microgrammes of adrenaline.

Unoperated rats								Hepatectomized rats			
Controls		Colchicine per 100 g.						Controls		Colchicine per 100 g.	
		0.1 mg.		0.5 mg.		1.0 mg.				0.1 mg.	
Total	Per 100 g. rat	Total	Per 100 g. rat	Total	Per 100 g. rat	Total	Per 100 g. rat	Total	Per 100 g. rat	Total	Per 100 g. rat
43.5	18.1	31.5	17.0	21.5	14.1	13.0	11.8	26.2	13.7	12.2	6.4
31.0	13.2	47.5	26.4	30.7	21.8	6.2	4.7	24.0	12.0	8.7	5.4
30.0	15.0	39.0	24.4	22.1	17.0	12.5	11.3	29.0	16.0	12.3	6.8
20.0	11.0	30.0	17.6			15.1	10.8	29.5	15.2	13.6	8.5
34.0	13.5	34.5	17.7					29.0	19.7	13.5	7.9
38.5	22.0	67.9	20.5					31.2	16.3	11.5	6.8
50.5	25.2	65.7	22.0					29.6	16.6	11.0	6.5
45.3	23.8	69.6	24.4					38.0	22.4		
79.9	25.0	58.9	16.3					21.0	13.3		
56.2	16.5							30.5	23.6		
								36.0	15.2		
Mean	18.3		20.7		17.0		9.6		16.7		6.9
S.E.	±1.8		±1.3		±2.2		±1.7		±1.1		±0.4

for Higgins and Anderson showed that during this period there were frequent fluctuations in the fluid content of the liver, though its dry weight remained constant.

The finding that the DOPA-decarboxylase activity of the liver both of normal and hepatectomized rats was much reduced if the animals were previously given colchicine was unexpected. The reduction could not have been due to a direct inhibition of the enzyme by the drug, for colchicine added to the flask did not reduce activity, although it might have been slowly changed in the body to some compound which did inhibit the enzyme. The other possibility is that colchicine has a slow toxic action, which causes a loss of coenzyme or apoenzyme. We have shown that there was some loss of coenzyme, for the enzymic activity of the liver from treated rats was in part reactivated by the addition of synthetic codecarboxylase. From this experiment, however, it did not appear that the action of colchicine could be completely explained by its effect on the coenzyme. It seems likely, therefore, that colchicine, or some metabolic product of it, has a toxic effect on the liver cell which causes a loss of enzyme protein. In this connexion, it is interesting to note that colchicine did not affect the activity of amine oxidase when given to normal or hepatectomized animals.

We have shown that the treatment of rats with colchicine much reduces the pressor amine content of their adrenals. Such a reduction could be due (i) to an increased output from the gland such that the rate of formation was not sufficient to make up the loss, (ii) to a decreased rate of formation in the gland while the out-

put remained constant, or (iii) to a combination of increased output and decreased formation. It is interesting to consider these three possibilities in turn. In the first place, although it is not known whether colchicine increases the rate of liberation of adrenaline from the adrenals, it may well do so, for it is known to cause "stress," i.e. it produces an increased output of ACTH from the pituitary (Wolfson quoted by Vogt, 1951), and substances which have this action might be expected to cause sympathetic hyperactivity. Drugs which cause stress do not necessarily reduce the pressor amines, for Anderson, Blaschko, Burn, and Mole (1951) showed that hexoestrol, which causes adrenal enlargement by acting on the pituitary, produced no such reduction. However, our results do not exclude the possibility that colchicine increases the rate of output of adrenaline so much that the normal rate of formation is not sufficient to prevent the content falling. The second possible explanation, that colchicine acts merely by slowing the formation of pressor amines, is interesting in view of our finding that it decreases the liver DOPA-decarboxylase and the suggestion by Blaschko (1939) that DOPA-decarboxylase may play a part in the biosynthesis of adrenaline, a suggestion that is supported by the work of Blaschko, Burn, and Carter (1951). Fig. 6, derived from Tables I and

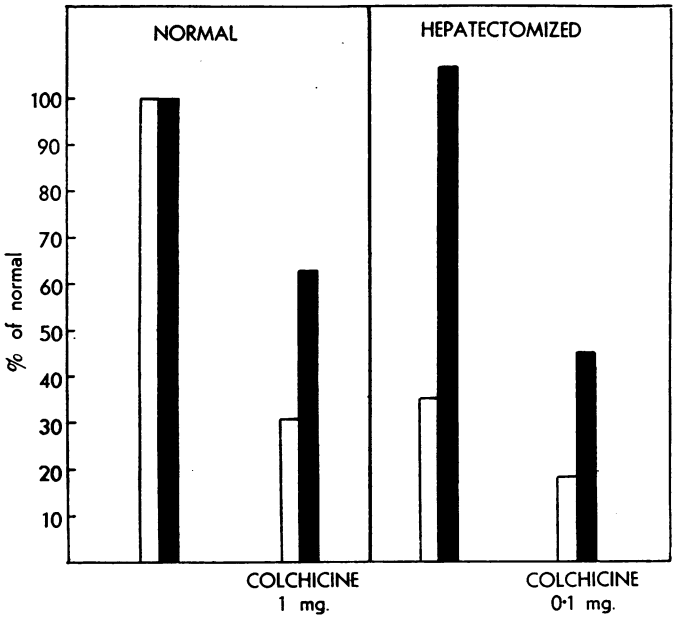

FIG. 6.—The effect of colchicine on the liver DOPA-decarboxylase □ and the pressor amine content of the adrenals ■ of normal and hepatectomized rats.

II, sets out side by side the effects of colchicine on enzyme activity and pressor amine content. It will be seen that in normal rats 1.0 mg. colchicine per 100 g. reduced the liver enzyme activity by nearly 70 per cent and the pressor amines by 40 per cent. Simple hepatectomy, however, which also reduced the enzyme content by nearly 70 per cent, had no effect whatever on the pressor amines. Colchicine cannot therefore have reduced them merely by its action on the liver enzyme. The occurrence of DOPA-decarboxylase in the adrenal of the ox (Langemann, 1951)

raises the possibility that it is this enzyme rather than the liver enzyme which is concerned with adrenaline formation. If this were so in the rat, it would be easy to understand why hepatectomy, which merely affected the liver enzyme, had no effect on the pressor amines, while colchicine, which might well reduce the activity of any possible adrenal enzyme, lowered them. The third explanation, that colchicine both interfered with adrenaline formation by reducing liver DOPA-decarboxylase and at the same time increased the rate of output from the adrenals, is also possible. Finally, colchicine may affect, directly or indirectly, some other reaction involved in the biosynthesis of adrenaline.

SUMMARY

1. The amine oxidase and L-DOPA-decarboxylase activity of liver was determined after partial hepatectomy in rats. During the first two days after operation there was no change in the total enzymic activity, but the liver increased in weight. The concentration of enzymes in the liver therefore fell. After the second day the total enzyme activity increased, and reached about 80 per cent of its original value 20 days after the operation.

2. Colchicine (0.1 mg. per 100 g.), given to hepatectomized rats 24 hours after operation, had no effect on the total amine oxidase activity of the liver. The total DOPA-decarboxylase activity was, however, reduced to about 50 per cent of its normal value. When colchicine was given in this and higher doses to normal rats also, the liver DOPA-decarboxylase was reduced. Colchicine itself did not inhibit the enzyme *in vitro*. The reduced activity could be only slightly increased by addition *in vitro* of synthetic pyridoxal phosphate (codecarboxylase).

3. Colchicine (0.1 mg. per 100 g.), given to hepatectomized rats, reduced the pressor amine content of the adrenals to about 40 per cent of the normal value. When given to normal rats, a large dose of colchicine (1.0 mg. per 100 g.) also reduced the pressor amines, but smaller doses (0.05 mg. and 0.1 mg.) had no effect.

4. These findings are discussed with particular reference to theories of the biosynthesis of adrenaline.

REFERENCES

Anderson, C. T., Blaschko, H., Burn, J. H., and Mole, R. H. (1951). *Brit. J. Pharmacol.*, **6**, 342.
Blaschko, H. (1939). *J. Physiol.*, **96**, 50 *P*.
Blaschko, H., Burn, J. H., and Carter, C. W. (1951). *J. Physiol.*, **115**, 37 *P*.
Blaschko, H., Carter, C. W., O'Brien, J. R. P., and Sloane Stanley, G. H. (1948). *J. Physiol.*, **107**, 18 *P*.
Brues, A. M., Drury, D. R., and Brues, M. C. (1936). *Arch. Path. Lab. Med.*, **22**, 658.
Brues, A. M., and Jackson, E. (1937). *Amer. J. Cancer*, **30**, 504.
Cotzias, G. C., and Dole, V. P. (1951). *J. biol. Chem.*, **190**, 665.
Higgins, G. M., and Anderson, R. M. (1931). *Arch. Path. Lab. Med.*, **12**, 186.
Langemann, H. (1951). *Brit. J. Pharmacol.*, **6**, 318.
Sloane Stanley, G. H. (1949). *Biochem. J.*, **45**, 556.
Vogt, M. (1951). *J. Physiol.*, **113**, 129.