Studies on the Liver Catalase of Normal and Cancerous Rats

By H. WEIL-MALHERBE* and R. SCHADE, *Departments of Physiology and Pathology, Medical School, King's College, University of Durham, Newcastle upon Tyne*

(*Received* 30 *December* 1947)

Although the investigation of the systemic effects of malignant tumours is likely to constitute a fruitful approach to the cancer problem, the difficulty has always been the distinction between true cancer effects and those of a secondary nature, due to the concomitant destruction or impairment of vital organs and their functions, to necrosis, infection or suppuration, to haemorrhage and similar accessory factors. One of the least ambiguous examples of a specific systemic cancer effect is the diminution of liver catalase activity in a tumour-bearing organism.

This decrease was first described by Brahn (1916), who determined the catalase concentration in the livers of human beings who had died from various forms of cancer, and was recently extensively studied by Greenstein (1942) and his associates (Greenstein & Andervont, 1942, 1943; Greenstein, Andervont & Thompson, 1942). Their work, which was carried out on rats with transplanted subcutaneous tumours and on mice with transplanted, spontaneous or induced tumours of various kinds, established the following facts: (1) The decrease of liver catalase is progressive with the growth of the tumour. The activity may fall to one tenth or one twentieth of the normal value within 2 or 3 weeks after transplantation of a rapidly growing tumour. (2) The effect is reversible and the liver catalase activity returns to normal after excision or spontaneous regression of the tumour. Thus, in rats from which a transplanted hepatoma had been removed the normal liver catalase activity was restored almost completely after 24 hr. and fully after 48 hr. On reimplantation of the tumour the liver catalase activity dropped again and was again brought back to normal by a second excision. (3) Kidney catalase activity is depressed much less than that of liver; only in mice with spontaneous mammary tumours is it lowered as much as, or more than, liver catalase. Erythrocyte catalase is apparently not affected at all. (4) The fall of catalase activity in the liver is probably due to a decrease of catalase concentration. No evidence for the presence of a catalase inhibitor could be found in tumour extracts or in the liver or serum of tumour-bearing rats. (5) The effect is specific in a twofold sense: although a few other liver enzymes are also less active as a result of a tumour elsewhere, e.g. D-amino-acid oxidase (Westphal, 1943) or arginase (Greenstein, Jenrette, Mider & White, 1941; Weil, 1935), the reduction is less striking than that of catalase. The majority of liver enzymes are unaffected. On the other hand, as far as is known at present, the catalase effect is caused only by cancer. In particular, it is not simply due to the presence of growing tissue, since pregnancy or growing implants of embryonic tissue fail to induce it.

* Present address: Runwell Hospital, Wickford, Essex.

It is obviously of great interest to obtain a better understanding of this phenomenon. 'Excluding any form of radiation from the tumour as too far fetched, the tumour may produce the effects noted either by giving off some toxic product to the circulation, or else by abstracting from the circulation some material essential to the normal maintenance of the liver catalase' (Greenstein, 1947).

The experiments to be described were designed to test the hypotheses quoted above. The elaboration of toxic products by growing tumours has often been postulated. Such an assumption is not unreasonable in the face of the extensive degradation processes accompanying an uncontrolled aggressive growth, which not only destroys surrounding normal tissues, but in many cases outstrips its own blood supply. One might therefore expect protein breakdown products, such as proteoses and polypeptides, in the circulation, and there is indeed some evidence for this (Winzler & Burk, 1944). It has been suggested that the output of protein split products by the tumour leads to a state of generalized intoxication and is the cause of the cachexia and marasmus of cancer patients (Reding, 1938). The effect of parenteral injection of foreign protein and of protein split products on the liver catalase of normal rats was therefore investigated.

If the invasiveness and unco-ordinated growth of a tumour results in augmented protein breakdown, the high rate of new growth will, on the other hand, greatly increase the demand for nitrogenous building materials. It is well known that a growing tumour will satisfy these demands without any regard for the welfare of the host, and the decrease in the concentration of certain liver enzymes may be due to lack of supplies required for their resynthesis or even to an accelerated 'melting-down' process. The extent of the latter would depend upon the supply of nutrient material, and thus upon the diet.

In spite of the severe secondary anaemia often induced by cancer, the content of iron and copper in liver and spleen is usually above normal (Sandberg, Gross & Holly, 1942). It is improbable therefore that it is lack of these ions which is responsible for the decrease in either haemoglobin or catalase. It cannot be due to simple inanition either, since starvation of normal or tumour-bearing rats (Greenstein, Jenrette & White, 1941 *b*; Miller, 1947) leads to a fall of liver catalase activity only in proportion with the decrease

of liver proteins generally. The fall of liver catalase activity might, however, be caused by the exaggerated protein requirements of a rapidly growing tumour. Consequently, the progressive decline of liver catalase activity in two series of cancer-bearing rats maintained on a high- or a low-protein diet was studied. For the high-protein diet a content of 45% casein was chosen, as this is sufficient to ensure maximal growth, whereas the low-protein diet contained 8% casein, an amount adequate for the maintenance of body weight or even a moderate rate of growth in normal rats.

EXPERIMENTAL

Materials and methods

Animals. Rats of both sexes from two inbred strains, a black-hooded and a brown and white strain, were used at the age of 2–3 months, when they weighed *c.* 100–150 g. Their routine diet consisted of 'rat cubes' (North-Eastern Agricultural Co-operative Society, Aberdeen) with an approximate protein content of 20%, and of occasional supplements of green cabbage leaves. The low-protein and high-protein diets had the following composition:

	Low protein	High protein
Casein (%)	8	45
Maize starch (%)	72	35
Arachis oil (%)	15	15
Salts (Wesson, 1932) (%)	5	5

Water was added to the mixture to make a thick dough. The following vitamin supplements were dissolved in 100 ml. water: aneurin 6 mg., pyridoxin 6 mg., riboflavin 10 mg., calcium pantothenate 20 mg., inositol 200 mg., nicotinamide 200 mg., choline chloride 600 mg. The solution (5 ml./rat) was added daily to the water used for mixing with the food. Once weekly a few drops of cod-liver oil were added to the ration. The average amount consumed daily/rat was about 15 g. Drinking water was supplied *ad libitum*. The rats were put on the diet 1 week before their inoculation with the Jensen rat sarcoma.

Serum injections. Sheep serum was inactivated by heating to 56° for 0·5 hr. It was then passed through a bacteriological filter, sealed aseptically in ampoules and stored at 0°. Intraperitoneal injections of 2 ml. were given daily for 3–4 weeks, and were well tolerated. In animals killed only a few hours after injection the injected fluid was usually completely resorbed, and there was no sign of local reaction. Five rats surviving after 4 weeks therefore received an increased daily volume of 5 ml. for a further 15–30 days. The total amount injected thus varied from 45 to 210 ml. of serum per rat. Since the results showed no variation according to the total quantity or period of injections they were grouped together.

Peptone injections. A 10% solution of a commercial sample of bacteriological peptone was adjusted to pH 7·25 (glass electrode), and subsequently sterilized and stored in the same way as serum. It gave a voluminous precipitate on addition of saturated $(NH_4)_2SO_4$ solution, and presumably contained proteoses. Two ml. were administered daily by intraperitoneal injection for 40–83 days, corresponding to a total of 80–166 ml. of peptone solution. Here, again, the results were grouped together, since no difference was apparent between animals killed at the beginning and those killed at the end of the period. The peptone injections were not so well tolerated as the serum injections, resulting in loss of weight and listlessness. Reddening and hyperaemia of the peritoneal membranes were frequently observed at autopsy.

Transplantation of tumours. The Jensen rat sarcoma (J.R.S.) was used for transplantation. The original tumour was obtained from the Imperial Cancer Research Fund Laboratories. Regressions were frequent in the beginning, but diminished after a few passages in our strains. Although a few experiments were performed on rats bearing slow-growing or regressing tumours, these were not included in the results.

Preparation and standardization of liver extract. After decapitation and exsanguination of the animal, the liver was removed and a weighed amount (*c.* 7 g. if available) was thoroughly homogenized with a little distilled water in a glass homogenizer (Potter & Elvehjem, 1936). After adding more water, in all three times the weight of liver used, the homogenate was left in the refrigerator overnight and was then centrifuged at 2500 r.p.m. for 5 min. The supernatant liquid was diluted to a volume proportionate to the weight of tissue taken, i.e. 100 ml. for 7 g. of liver.

It is advisable to adjust the extracts to a definite protein content. For this purpose Greenstein, Jenrette & White (1941*a*) estimated the total N of the extract. We found it more convenient to use the rapid colorimetric estimation of protein with Folin & Ciocalteu's reagent. All extracts were accordingly diluted to a certain value of 'protein tyrosine', obtained by subtracting the 'non-protein tyrosine' from the 'total tyrosine'. The total tyrosine was estimated in 0·2 ml. of extract; after addition of 5N-NaOH (2 ml.) and reagent (3 ml.), the solution was made up to 50 ml. For the estimation of non-protein tyrosine, 5 ml. of a tungstic acid filtrate of the extract were used. The light absorption was determined photoelectrically with a tricolour-red filter and calibrated with a tyrosine standard. As shown in Table 1 there is a constant ratio between the protein tyrosine and the protein N.

Table 1. *Content of tyrosine and nitrogen in liver extracts of normal rats*

Extract	Total tyrosine (mg./ml.)	Total N (mg./ml.)	N/tyrosine
1	1·40	2·910	2·08
2	1·15	2·520	2·19
3	1·60	3·740	2·34
4	1·60	3·612	2·26
5	1·13	2·316	2·08
			Mean **2·19**
Extract	Protein tyrosine	Protein N	N/tyrosine
1	1·280	2·826	2·21
2	1·060	2·348	2·21
3	1·442	3·491	2·42
4	1·479	3·398	2·30
5	1·040	2·167	2·13
			Mean **2·25**

Estimation of catalase activity. The catalase activity was determined by measuring the rate of H_2O_2 decomposition colorimetrically with titanium sulphate (cf. Eisenberg,

1943). This reagent was prepared as follows: Anhydrous titanium dioxide (2 g.) was heated on a sand bath with 200 ml. of conc. H_2SO_4 at 155–165° until a clear solution was formed (usually 3–4 hr.). After cooling, the solution was poured into distilled water, diluted to 1 l. and filtered through Whatman no. 5 filter paper. One vol. of the reagent was mixed with 9 vol. of H_2O_2 solution containing 0·005–0·05 mg./ml., and the ensuing colour, which was stable for at least 24 hr., was measured photoelectrically with a blue filter. A calibration curve was determined with a solution of H_2O_2, standardized by $KMnO_4$ titration.

If the concentration of phosphate buffer in the yellow solution of pertitanic acid is higher than 0·01 M, a turbidity may appear. This can easily be avoided by the addition of 0·1 vol. of 10 N-H_2SO_4 without in any way affecting the colour development. In practice this was seldom necessary, since the H_2O_2 concentration was usually sufficiently high to require further dilution.

The catalase concentration of a liver extract was tested at three different concentration levels at 0°. The H_2O_2 concentration was 0·02 N in the majority of experiments; the solution also contained 0·04 M-phosphate buffer, pH 6·8. At time $t=0$, 1 ml. of enzyme solution of required strength was added to 50 or 100 ml. of H_2O_2-phosphate buffer solution in an ice bath. After 3, 6, 9 and 12 min., sometimes also after 1 min., samples of 5 or 10 ml. were withdrawn and rapidly mixed with 0·1 vol. of 30% (w/v) trichloroacetic acid. A sample of the filtered solution was used for the colorimetric assay. The initial value of H_2O_2 concentration was determined after adding 1 ml. of enzyme solution to a sample containing 50 ml. of H_2O_2-buffer solution + 5 ml. of 30% trichloroacetic acid and filtering. The monomolecular reaction constant was calculated by the formula

$$k=\frac{2\cdot3}{t}\log\frac{[H_2O_2]_{\text{initial}}}{[H_2O_2]_{t\,\text{min.}}}.$$

Haemoglobin. This was estimated by the Haldane method.

RESULTS

Effects of high- and low-protein diets on growth. The growth rate of tumour-bearing rats on the high- or low-protein diet was determined by weighing them before transplantation, and again at death, after excision of the tumour which was weighed separately. From the data in Table 2 the following facts emerge: (1) The growth of the tumour is accompanied by a fall of the body weight of the host even on a high-protein diet, but this is significantly more severe in the rats on the low-protein diet. (2) The rate of tumour growth is significantly greater on the high- than on the low-protein diet. (3) The faster rate of tumour growth more than compensates for the loss of body weight in the high-protein series, whereas the animals on the low-protein diet show not only a fall of body weight, but also of total weight. (4) If tumour weight is plotted against days, an exponential curve is obtained, rising steeply as the tumours become older. If log (tumour weight) is plotted against days the points are grouped about a straight line (Fig. 1), the slope of which can be calculated by the method of least squares. The slope of the high-protein curve is significantly steeper than that of the low-protein curve, indicating a more rapid rate

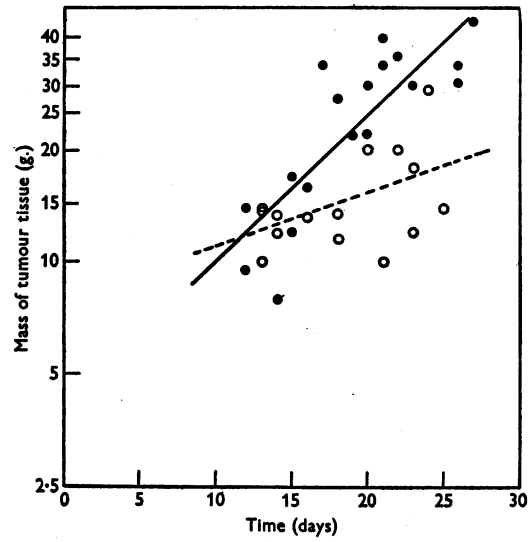

Fig. 1. Regression (statistical) of tumour weight with time. High-protein diet: dots and full line. Low-protein diet: circles and broken line. The straight lines are the linear regression lines.

Table 2. *Effect of high- and low-protein diets on body weight and tumour growth*

(Figures represent mean values and their standard errors.)

	Low-protein diet	High-protein diet	P*
No. of observations	15	18	—
Days after transplantation	18·5	19·1	—
Final body weight (% of initial value)	77·0 ±2·64	90·5 ±2·60	<0·01
Tumour growth (g./days)	0·82±0·051	1·30±0·091	<0·01
Total weight at death (body + tumour; % of initial value)	91·5 ±2·28	109·1 ±1·71	<0·01
Regression coefficients			
Log (tumour weight) on days	0·0139±0·00688	0·0393±0·00675	0·015
Log (body weight)/(tumour weight) on days	−0·0315±0·00629	−0·0494±0·00473	0·03

* P = probability of difference being due to chance.

Table 3. *Protein tyrosine content of liver extracts*

Exp.	Days after transplantation (mean)	No. of observations	Protein tyrosine (mean) (mg./ml.)	Standard error of mean
	Normal rats			
Cube diet	—	17	0·922	0·0178
High-protein diet.	—	6	0·975	0·0375
Low-protein diet	—	8	0·833	0·0202
Injected with sheep serum	—	10	0·896	0·0200
Injected with peptone	—	5	0·923	0·0285
	Tumour-bearing rats			
Cube diet	22·70	19	0·710	0·0227
High-protein diet	19·26	20	0·697	0·0160
Low-protein diet	18·14	14	0·655	0·0182

of growth. (5) When the logarithm of the ratio (body weight)/(tumour weight) is plotted against days and the linear regression curve calculated

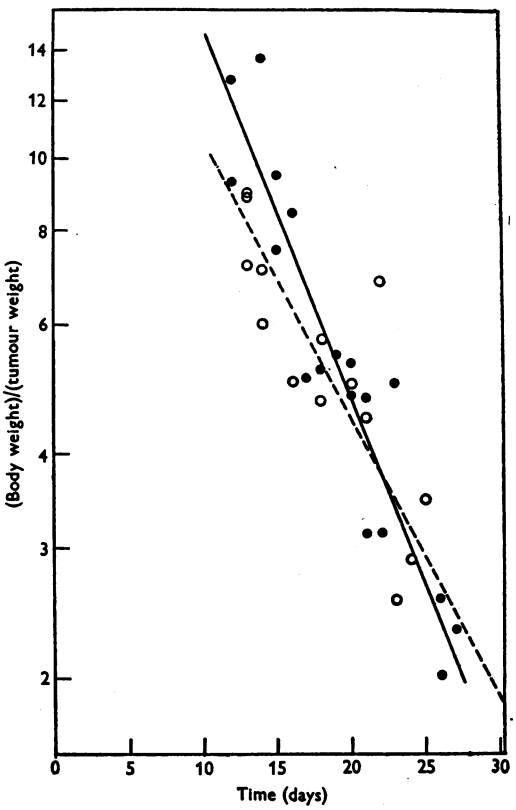

Fig. 2. Regression of ratio (body weight)/(tumour weight) on days after transplantation. High-protein diet: dots and full line. Low-protein diet: circles and broken line. The straight lines are the linear regression lines.

(Fig. 2), it is found that the difference between the high- and low-protein series, though still probably significant, is less than that between the curves representing tumour growth rates. This indicates that the faster tumour growth on the high-protein diet is to some extent balanced by a smaller loss of body weight.

Changes in protein tyrosine of liver. (*a*) *Normal rats.* The concentration of protein tyrosine in liver extracts of normal rats on the standard diet showed very little individual variation. It was not significantly raised either by a high-protein diet or by injections of serum or peptone (Table 3). On the other hand, there was a slight decrease in the rats on the low-protein diet, the difference from the standard series being statistically significant ($P < 0·01$). The animals on the high- or low-protein diets which were used for these analyses had previously been inoculated with J.R.S., but the tumours had failed to grow or had regressed. The analyses were performed 4–5 weeks after the start of the diets and not less than 1–2 weeks after the complete disappearance of tumours.

(*b*) *Tumour rats.* The presence of a growing tumour invariably caused a considerable decrease of the protein-tyrosine concentration of liver extracts, which was not significantly affected by the protein level of the diet.

Table 4. *Haemoglobin concentration*

	No. of observations	Mean Hb (%)
Normal rats		
Cube diet	8	79·6
High-protein diet	6	85
Low-protein diet	8	76·6
Tumour rats		
Cube diet	11	49·7
High-protein diet	16	66·1
Low-protein diet	14	54·6

Haemoglobin. Estimations of haemoglobin were carried out in a number of cases and the results are shown in Table 4. There is a marked drop in tumour-bearing rats, as is well known (cf. Taylor & Pollack, 1942). Whether the differences between the high- and low-protein series are significant seems doubtful in view of the high margin of error of the method.

There were, however, indications of a correlation between the degree of secondary anaemia and the size and age of the tumour.

The catalase activity of liver extracts of normal and tumour-bearing rats. Catalase was measured at three concentration levels after 3, 6, 9 and 12 min. During this time the activity gradually diminished by 10–20%. In most experiments the enzyme concentrations were varied in the proportion of 1 : 2 : 4, the actual values depending on the presence of a tumour and its size. A value for the monomolecular reaction constant of *c.* 0·1 with the highest and of *c.* 0·025 with the lowest enzyme concentration was sought. With extracts of normal rats, final concentrations of 0·4–0·1 μg./ml. protein tyrosine were required; 1 ml. of extract containing 20–25 μg./ml. protein tyrosine was added to 50 ml. of substrate solution. With tumour rats, these concentrations had to be increased 5–10-fold. The H_2O_2 concentration in these experiments was 0·02 N throughout.

There was a very satisfactory proportionality of k (the reaction constant) with enzyme concentration (Table 5), unaffected by the relative substrate concentration. In a number of experiments the enzyme concentration was varied in the proportion of 1 : 10 : 100, to test for a possible inhibitory effect of serum or peptone injections which might have manifested itself only at high enzyme concentrations. But the fairly close proportionality of k with enzyme concentration, even over this extreme range, makes the existence of such an effect very unlikely. The ratio of substrate/enzyme concentration was kept constant here in order to avoid an unequal degree of enzyme inhibition by excess substrate.

The satisfactory proportionality between enzyme activity and concentration, at any rate over a limited range, makes it possible to express enzyme units in terms of activity and protein content. Catalase concentration has therefore been expressed in arbitrary units defined by the ratio k/mg. protein tyrosine/ml. The mean value of the results obtained with the three different enzyme concentrations was taken as the unit content of a given extract.

Catalase concentration in the liver of non-tumour rats. The concentration of catalase in the liver of normal rats is fairly constant if extracts of equal protein concentration are compared. It is not significantly changed either by high- or low-protein diets or by injections of peptone solutions (Table 6). However, injection of sheep serum leads to a significant increase of catalase concentration. Not only the difference between serum and standard series, but also that between serum and peptone series, is significant ($P < 0·01$ and $P = 0·01$ respectively). This is the more remarkable as the protein concentration of liver extracts was not raised by serum injections. On the other hand, although rats on the low-protein diet show a decrease of liver protein, the ratio between protein and catalase concentrations remains constant.

Since there was no essential difference between them, the first four experiments of Table 6 were grouped together to provide a standard of comparison with the catalase concentration in the liver of tumour rats.

Catalase concentration in the liver of tumour-bearing rats. Where our experiments covered the same ground as those of Greenstein and his associates the results were in full agreement with theirs. A fall in catalase activity was found, which was progressive with the growth of the tumour (in extreme cases, to

Table 5. *Proportionality of the monomolecular reaction constant k with enzyme concentration*

Series	No. of observations	Protein tyrosine (mg./ml. liver extract)	H_2O_2 concentration (N)	Mean value of k after time (min.) 3	5	12
Normal rats, cube diet	17	0·02	0·02	0·1043	—	0·0906
		0·01	0·02	0·0520	—	0·0460
		0·005	0·02	0·0276	—	0·0232
Normal rats, injected with serum or peptone	8	0·25	0·2	1·20	1·10	—
		0·025	0·02	0·178	—	0·147
		0·0025	0·002	0·0202	—	0·0158

Table 6. *Concentration of liver catalase in normal rats*

(Values in arbitrary units/ml.; see text.)

Exp.	No. of observations	Catalase concentrations (mean values) ± standard errors of mean after time (min.) 3	12
Cube diet	17	5·21 ± 0·316	4·57 ± 0·267
High-protein diet	5	5·55 ± 0·438	4·34 ± 0·404
Low-protein diet	8	5·20 ± 0·190	4·28 ± 0·163
Injected with peptone	5	6·01 ± 0·353	4·93 ± 0·280
Injected with serum	10	7·58 ± 0·427	6·24 ± 0·282
First 4 experiments combined	35	5·37 ± 0·301	4·52 ± 0·265

only 5% of normal). There were, however, large individual variations, even when similar stages of tumour growth were compared.

If a correlation is to be sought between the size of the tumour and the concentration of liver catalase, the absolute size of the tumour might be regarded as of less importance than the tumour size relative to the size of the host, as expressed by the ratio (body weight)/(tumour weight). If catalase concentrations are plotted against this ratio, an exponential curve is again obtained, and the points are grouped along a straight line when plotted on a semi-logarithmic scale (Fig. 3). In spite of the wide scatter, the regressions of catalase concentration on the ratio of (body weight)/(tumour weight) are significantly different from zero in the high- and low-protein experiments. In the experiment with rats on the standard diet the (statistical) regression is hardly significant ($P = 0{\cdot}09$). This is probably because the points were not sufficiently spread out along the x-axis.

It is quite obvious from an inspection of Fig. 3, and is confirmed by statistical analysis of the regression coefficients (Table 7), that the effect of tumour growth on the concentration of liver catalase is not significantly influenced by the level of protein in the diet. This is so not only in the case of large tumours, when it may be doubtful whether even a high-protein diet will sufficiently meet the requirements, but also in the initial stages where an effect might be expected if lack of protein were a decisive factor.

If the concentration of liver catalase is compared in rats with a similar ratio of (body weight)/(tumour weight), but with a different growth rate, it appears that it is on the whole lower when the tumours are growing slowly or are regressing than in the case of early, fast-growing tumours (Table 8). This suggests that not only the relative size, but also the age of the tumours is of importance.

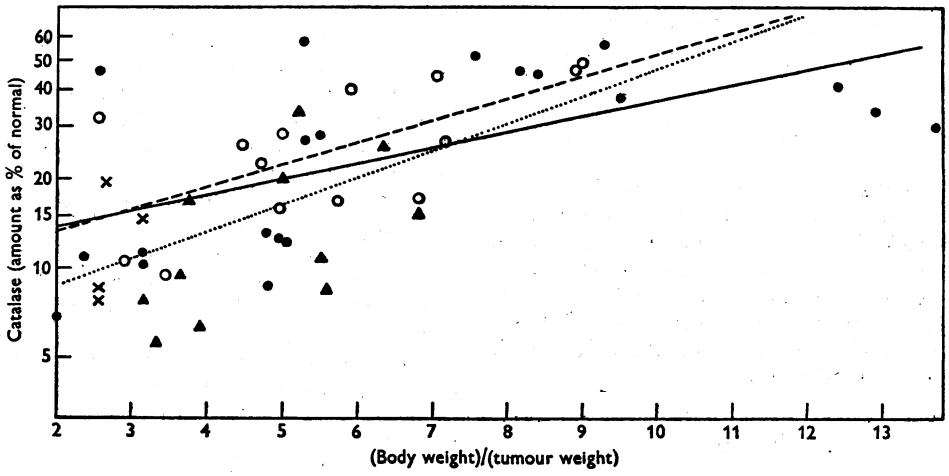

Fig. 3. Regression of liver catalase concentration (in % of normal concentration) on ratio (body weight)/(tumour weight). Standard diet: triangles and dotted line. Standard diet and serum injections: crosses. High-protein diet: dots and full line. Low-protein diet: circles and broken line. The straight lines are the linear regression lines.

Table 7. *Concentration of liver catalase in tumour-bearing rats*

Series	No. of observations	(Body weight)/(tumour weight) (mean)	Days after transplantation (mean)	Catalase units (% normal) (mean)	Regression coefficient of catalase concentration on (body weight)/(tumour weight)
Cube diet	15	4·20	22·14	13·95	0·0747±0·0408
High-protein diet	20	6·53	19·05	29·22	0·0517±0·0165
Low-protein diet	14	5·61	18·14	27·65	0·0734±0·0254

In view of the definite increase of liver catalase observed in normal rats after intraperitoneal injections of sheep serum, the effect of serum injections on tumour-bearing rats was of interest. Four rats (marked × in Fig. 3) were given daily intraperitoneal injections of 5 ml. of sheep serum, starting 11 days after transplantation when the tumours were easily palpable. The injections were continued for 8–10 days, so that a total amount of 40–50 ml. was injected. There was no obvious effect on either the rate of tumour growth or the concentration of liver

catalase. Two more series each consisting of five rats received serum injections from the fourth and fifth day after transplantation onwards. In all ten animals regression of the tumours occurred. In two control series the tumours grew in 3 out of 5 and in 3 out of 3 animals. It would be premature to draw any conclusions from the small number of animals in these experiments, especially in view of a tendency towards regression in the controls, but the results suggest an inhibitory effect of early serum injections on the growth of transplanted J.R.S. This point is being further investigated.

Table 8. *Concentration of liver catalase in tumour-bearing rats*

Days after transplantation	(Body weight)/(tumour weight)	Catalase concentration (% normal)
	Fast growing, early tumours	
13	8·9	46·9
13	9·0	49·7
12	9·3	57·3
12	12·9	33·8
14	7·06	44·4
	Slow growing or regressing tumours	
21	29·0	24·3
23	11·6	27·6
23	12·2	15·9
26	7·15	10·3
27	14·3	35·8

DISCUSSION

The fact that the presence of a growing tumour leads to a progressive fall of the concentration of liver catalase far in excess of the lowering of liver proteins generally need not necessarily be inconsistent with an unspecific inhibition of protein synthesis such as would result from the appropriation of available amino-acids by the tumour. If the rate of disintegration and regeneration were much faster for catalase than for other liver enzymes, the specific effect on catalase could be interpreted as the outcome of an unspecific inhibition of protein synthesis. There are, indeed, indications that liver catalase has a short life: (1) it has a high rate of wastage during enzymic activity; (2) a high rate of resynthesis after removal of a transplanted tumour; (3) in contrast to erythrocyte catalase, it contains a verdohaemochromogen group which, as Lemberg & Legge (1943) suggest, is due to an *in vivo* oxidation.

The experiments on the effect of high- and low-protein diets have provided no support for this mechanism. If a shortage of amino-acids were the cause, one would have expected at least a delay in the decline of catalase concentration as a result of the high-protein feeding. That the fall of body weight is considerably less severe on the high- than on the low-protein diet shows that the higher protein level is not entirely ineffectual, and the lack of response of the catalase concentration suggests that other causes than protein shortage are operating. This conclusion, however, is not yet final. An effect might appear if the rate of protein synthesis could be speeded up, e.g. by parenteral administration of essential amino-acids in high dosage. Alternatively, an excess of one or more particular amino-acids may be required. The observation that serum injections raised the level of catalase concentration in normal livers, whereas peptone injections were ineffective, may point in this direction. Peptone is particularly deficient in tryptophan, and it would be interesting therefore to study the effect of massive doses of this amino-acid.

The possibility that the lowering of catalase concentration in the liver is due to a toxic principle elaborated by the tumour and given off into the circulation also deserves further study. It is true that Greenstein (1943) found no indication for the presence of a catalase inhibitor in tumour tissue or in other tissues of cancerous animals. But the possible existence of an inhibitor of catalase synthesis or a catalyst of catalase destruction has not yet been exhaustively explored. Our experiments show that prolonged parenteral injection of foreign protein or of protein breakdown products does not duplicate the tumour effect in normal rats. A prolonged treatment of normal rats with tumour extracts might now be attempted. Greenstein (1943) carried out some experiments on these lines, but he gave only one injection of 1 or 2 ml. of extract, whereas it may be necessary to extend the injections over a period of several weeks. Moreover, his extracts, which were prepared with saline, were probably devoid of nucleoproteins, and it is feasible that the toxic factor is associated with this fraction.

SUMMARY

1. The concentration of liver catalase in normal rats and in rats bearing a transplanted Jensen rat sarcoma was determined. The rats were maintained on one of three diets: (*a*) a standard diet of rat cubes (20 % protein), (*b*) a high-protein diet (45 % casein), (*c*) a low-protein diet (8 % casein). Additional experiments were performed on rats which received daily intraperitoneal injections of sheep serum or of a 10 % solution of peptone for several weeks.

2. The high-protein diet caused a significantly faster growth of the tumours and a smaller loss of body weight in tumour-bearing rats than the low-protein diet. The protein content of the liver was slightly reduced in normal rats on the low-protein diet; it was considerably lowered in all tumour-bearing rats, irrespective of diet.

3. The catalase concentration in normal rat liver extracts of equal protein content was not significantly changed, either by different protein levels in the diet or by peptone injections. It was raised, however, by serum injections.

4. In confirmation of the work of Greenstein and his group (1941–3) it was found that the growth of a transplanted tumour leads to a fall of liver catalase concentration which, in extreme cases, may reach 5 % of normal. There was a very marked individual scatter even in similar stages of tumour growth.

5. The catalase concentration when plotted against (body weight)/(tumour weight) was found to follow an exponential curve.

6. The protein level of the diet had no significant effect on the course of the process, even in the initial stages. Serum injections had no effect on tumour-bearing rats when started 11 days after transplantation. When started 4 or 5 days after transplantation they were followed by regression of the tumour.

We are indebted to Dr B. Pullinger, Imperial Cancer Research Fund, Mill Hill Laboratories, London, for specimens of the Jensen rat sarcoma.

REFERENCES

Brahn, B. (1916). *S.B. preuss. Akad. Wiss.* p. 478.
Eisenberg, G. M. (1943). *Industr. Engng Chem.* (Anal. ed.), **15**, 327.
Greenstein, J. P. (1942). *J. nat. Cancer Inst.* **2**, 525.
Greenstein, J. P. (1943). *J. nat. Cancer Inst.* **3**, 397.
Greenstein, J. P. (1947). *Biochemistry of Cancer.* New York: Academic Press.
Greenstein, J. P. & Andervont, H. B. (1942). *J. nat. Cancer Inst.* **2**, 345.
Greenstein, J. P. & Andervont, H. B. (1943). *J. nat. Cancer Inst.* **4**, 283.
Greenstein, J. P., Andervont, H. B. & Thompson, J. W. (1942). *J. nat. Cancer Inst.* **2**, 589.
Greenstein, J. P., Jenrette, W. V. & White, J. (1941*a*). *J. nat. Cancer Inst.* **2**, 17.
Greenstein, J. P., Jenrette, W. V. & White, J. (1941*b*). *J. nat. Cancer Inst.* **2**, 283.
Greenstein, J. P., Jenrette, W. V., Mider, G. B. & White, J. (1941). *J. nat. Cancer Inst.* **1**, 687.
Lemberg, R. & Legge, J. W. (1943). *Biochem. J.* **37**, 117.
Miller, L. L. (1947). *Fed. Proc.* **6**, 279.
Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
Reding, R. (1938). *Z. Krebsforsch.* **47**, 240.
Sandberg, M., Gross, H. & Holly, O. M. (1942). *Arch. Path.* **33**, 834.
Taylor, A. & Pollack, M. S. (1942). *Cancer Res.* **2**, 223.
Weil, L. (1935). *J. biol. Chem.* **110**, 201.
Wesson, L. G. (1932). *Science*, **75**, 339.
Westphal, U. (1943). *Hoppe-Seyl. Z.* **276**, 213.
Winzler, R. J. & Burk, D. (1944). *J. nat. Cancer Inst.* **4**, 417.

The β-Glucuronidase Activity of Ox Spleen and the Assay of β-Glucuronidase Preparations

By G. T. MILLS,* *Biochemistry Department, St Thomas's Hospital Medical School, London, S.E.* 1

(*Received* 7 *January* 1948)

The existence in animal tissues of an enzyme which hydrolyzes β-glucuronides was first reported by Sera (1915). In 1934, Masamune described a method of preparation of active extracts of this enzyme from ox kidney having pH optima for the hydrolysis of menthylglucuronide and phenylglucuronide in citrate buffers at 5·3 and 5·3–5·6 respectively.

Oshima (1934) reported data for the glucuronidase content of various tissues of the dog and the ox, rich sources being liver, spleen, kidney, ovary, testes and thymus. In 1936 Oshima introduced improvements into the method of Masamune (1934), which were claimed to give a purer preparation. For this preparation the pH optimum for the hydrolysis of menthylglucuronide in acetate buffer was quoted as 5·0–5·2. In the work reported up to that time, little or no data were presented concerning the degree of purification or the losses at each stage of the preparation of β-glucuronidase.

The first quantitative study of the purification of ox-spleen glucuronidase was reported by Fishman (1939*a*). By employing ammonium sulphate fractionation after preliminary treatment, Fishman achieved an eightfold concentration of the enzyme and a 140-fold purification. There was, however, an overall loss of 84 % by this method. Fishman (1939*b*) reported that the pH optima of his preparation in an acetate buffer were 5·0 for *l*-menthylglucuronide, 4·4 for borneolglucuronide and 4·3 for oestriol-glucuronide.

Florkin, Crismer, Duchateau & Houet (1942) also studied glucuronidase, and demonstrated the reversibility of the hydrolysis of borneolglucuronide *in vitro*.

* Present address: Biochemistry Dept., University of Glasgow.