On pancreatic lipase. IV. The action of serum on mice inoculated with malignant mouse tumour / by J.A. Shaw-Mackenzie.

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In previous communications(1) made in conjunction with Dr O. Rosenheim we noted that serum, although not possessing any fat-splitting (lipoclastic) action by itself, nevertheless increases the activity of pancreatic lipase.

In cases of carcinoma in the human subject and in certain pathological conditions, however, we made the observation that this reaction of the serum was increased in a remarkable way; whilst cholesterin exerted in all cases an inhibitory influence.

The present observations refer to the action of serum (1) of normal mice; (2) of mice which have been inoculated with mouse tumour and presenting progressive growths; (3) of mice which have recovered spontaneously from large growths; (4) of mice which have proved negative to inoculation with mouse tumour; (5) of mice which it is assumed have been rendered immune to subsequent inoculation with mouse tumour by previous injection with normal mouse-tissue emulsions; and (6) of mice which have been injected subcutaneously with some animal tissue extracts and substances.

Details of technique have been published already. Briefly, it may be explained that the lipoclastic action is estimated by the amount of decinormal potash used for the neutralisation of the fatty acids set free by pancreatic lipase acting on olive oil emulsion; the mixture being incubated at a temperature of 37° C. for a certain time, usually 18 hours.

Taking now normal mouse serum, the following table gives the results in a typical case; 0·7 c.c. of glycerin extract of pancreas with water, in the proportion of 1–2 and 2·5 c.c. of olive oil emulsion were mixed, and after incubation the amount of acid liberated was estimated.

TABLE I. *Average normal mouse serum.*

	c.c. $\frac{N}{10}$ KOH	Acceleration
Pancreatic extract alone on olive oil emulsion	6·1	—
Addition of serum to extract 0·1 c.c. ...	8·2	+2·1
0·25 ,, ...	9·2	+3·1
0·5 ,, ...	10·1	+4·0

In aged mice the figures appeared to be somewhat less, whilst in well-fed healthy mice, of average weight (16–17 grammes), the figures are somewhat higher, with an acceleration of 5·0 to 5·5 for 0·5 c.c. of added serum.

The following tables summarise the results, when in a similar way the action of the serum was investigated under the various conditions specified above.

TABLE II. *Serum of mice with inoculated progressively growing tumours.*

Serum added	Acceleration
0·1 c.c.	+6·7
0·25 ,,	+8·0
0·5 ,,	+11·8

These figures show the markedly increased acceleration which occurs with the serum of malignant mice.

TABLE III. *Serum of mice which have recovered spontaneously from large growths.*

	Serum added	Acceleration
(*a*) Time factor (?)	0·1 c.c.	+1·3
	0·25 ,,	+3·2
(*b*) Nodules present	0·1 c.c.	+6·0
	0·25 ,,	+7·0
	0·5 ,,	+11·3

In this list two results were obtained which at first sight appear to be contradictory, but which are satisfactorily explained if the circumstances are considered.

In the first set (*a*) the serum reaction had returned apparently to normal; in the second set (*b*) the reaction was high and similar to that in mice with tumour growths.

In explanation it may be noted that in the first set the time which had elapsed since recovery was not known, and the reaction may have passed off in process of time. Examination of the serum in mice definitely known to have long recovered has not been performed as yet.

In the second set, though the mice had apparently recovered, it was found on post mortem examination that small nodules or diminished tumours still existed, subcutaneously, at the site of inoculation.

TABLE IV. *Serum of mice which have proved "negative" to inoculation with mouse tumour.*

	Serum added	Acceleration
(*a*) Time factor (?)	0·1 c.c.	+7·0
	0·25 ,,	+9·2
	0·5 ,,	+11·6
(*b*) 2½ months elapsed	0·1 c.c.	+2·7
	0·25 ,,	+4·1
	0·5 ,,	+6·3

Two results were obtained here also, and the surprising observation made that increased acceleration occurred in such cases.

In the first set (*a*) the figures are the same, practically, as in mice with tumour growths.

In the second set (*b*) the figures showed an increase over the normal but not reaching the high figures in the former.

In the first set the time since inoculation which proved negative was not noted, and examination of early negatives has not been made at present.

In the second set a known period of 2½ months had elapsed, and the reduced figures suggest that the reaction was passing off.

TABLE V. *Immunisation.*

	Serum added	Acceleration
Spleen	0·1 c.c.	+5·4
	0·25 ,,	+8·5
	0·5 ,,	+11·6
Liver	0·1 c.c.	+5·7
	0·25 ,,	+9·4
	0·5 ,,	+13·7
Testis	0·1 c.c.	+3·9
	0·25 ,,	+5·5
	0·5 ,,	+8·7

Immunisation. Various observers have demonstrated the fact that if an injection of certain normal mouse tissues has previously been made, the mice are rendered immune, absolutely, or in some degree, to subsequent inoculation with mouse tumour. Examination of the serum in regard to the lipoclastic reaction, therefore, after injection of some of the tissues which confer immunity, is of special interest.

Mouse spleen, liver, and testis emulsion have been used, so far, and an increase of lipoclastic acceleration occurred in each case. (See Table V.)

TABLE VI. *Animal tissue extracts and substances.*

	Serum added	Acceleration
Glycerin extract of duodenum (pig) 1–4 H_2O :		
(*a*) After 24 hours	0·1 c.c.	+2·3
	0·25 ,,	+4·2
	0·5 ,,	+4·7
(*b*) After 3 days	0·1 c.c.	+5·0
	0·25 ,,	+8·2
	0·5	+11·9
Sod. oleate 0·8 %:		
(*a*) After 24 hours	0·1 c.c.	+1·9
	0·25 ,,	+2·5
	0·5 ,,	+2·5
(*b*) After 3 days	0·1 c.c.	+3·9
	0·25 ,,	+6·3
	0·5 ,,	+10·3
Sod. cholalate 0·5 %:		
(*a*) After 24 hours	0·1 c.c.	+2·2
	0·25 ,,	+3·7
(*b*) After 3 days	0·1 c.c.	+5·0
	0·25 ,,	+7·1
	0·5 ,,	+10·8
Lipase[2] residue 1 % 1–4 H_2O :		
(*a*) After 24 hours	0·1 c.c.	+2·1
	0·25 ,,	+2·5
	0·5 ,,	+2·6
(*b*) After 3 days	0·1 c.c.	+4·7
	0·25 ,,	+7·3
	0·5 ,,	+10·5
Acid meta protein from white of egg :		
After 3 days	0·1 c.c.	+3·5
	0·25 ,,	+6·9
	0·5 ,,	+10·1
Acid meta protein from rabbit's muscle :		
After 3 days	0·1 c.c.	+4·8
	0·5 ,,	+11·9
Alkali meta protein from white of egg :		
After 3 days	0·1 c.c.	+3·1
	0·25 ,,	+4·2
	0·5 ,,	+6·8

TABLE VI (*continued*).

	Serum added	Acceleration
Boiled protein from white of egg :		
After 3 days	0·1 c.c.	+3·1
	0·25 ,,	+4·2
	0·5 ,,	+6·3
Cholesterin : (inhibitory)		
After 3 days	0·1 c.c.	+1·1
(One experiment only).	0·25 ,,	+2·2
	0·5 ,,	+2·9

Table VI shows the results obtained from serum of mice injected subcutaneously with animal tissue extracts and substances.

Although dosage has not been pushed to the full, it will be seen that acceleration in such instances is marked. How far immunisation is achieved by these means has not as yet been investigated.

An important feature was brought to light, namely that the time factor is of importance. Whilst no result or even a decrease was observed on examination of the serum 24 hours after injection, the acceleration was evident on examination after 3 days. It would appear thus that there is a negative and positive phase for substances other than bacterial vaccines.

It may be further stated that in inoculated mouse tumour and for animal tissues and extracts, in which the lipoclastic increased acceleration was observed, an increased antitryptic action of the serum was also obtained.

Further inquiries are in progress into the effects of the above substances; and of the various proteins of different tissues or of the tumour growth itself.

Conclusions. The facts that the lipoclastic acceleration of the serum in (1) the various phases of tumour inoculation and growth in mice; (2) in cases of spontaneous recovery; (3) in mice "negative" to inoculation; and (4) in animals presumably rendered immune to inoculation by certain mouse tissues, suggest that lipoclastic acceleration is one factor in the natural defensive or protective processes of the body and in the induced resistance or immunisation to inoculated tumour in mice.

In continued examination of the serum from blood in cases of malignant disease in the human subject, I have at present results in two cases in which examination of the serum was made after recovery. These results coincide with those in mice. In both cases, one of

recurrent carcinoma and the other of inoperable sarcoma, recovery was apparently complete, and the lipoclastic acceleration continued to exist after four years in the first case and three years in the second.

Lipoclastic acceleration is, however, not specific to cancer, but is found also in other pathological conditions.

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