Table 1. AMOUNT OF DNA REQUIRED FOR THE NEUTRALIZATION OF UNITS OF SERUM ANTIBODIES CONTAINED IN DIFFERENT SERA*

N/N	Source of serum	Diagnosis	The neutralizing dose of fDNA (γ)	Ratio of neutralizing doses of different preparations of DNA to neutralizing dose of formalin-treated DNA: nDNA / fDNA	dDNA / fDNA	apurDNA / fDNA	apyrDNA / fDNA
1	Guinea-pig	Normal	0·002	>1,000	125	—	—
2	,,	,,	0·002	>1,000	125	—	—
3	,,	,,	0·001	>1,000	250	—	—
4	,,	,,	0·004	>1,000	250	—	—
5	Patient K.	SLE	0·008	>1,000	66	No*	No*
6	,, M.	Discoid LE	0·008	>1,000	125	—	—
7	,, E.	Scrofuloderma	0·004	>1,000	250	—	—
8	,, P.	Werlhof's disease	0·008	>1,000	250	—	—
9	,, Ku.	SLE	0·008	160	4	1,400	No*
10	,, D.	,,	0·002	160	4	2,700	54,000
11	,, Ko.	,,	0·008	160	4	40	No*
12	,, Mo.	,,	0·004	80	4	80	130,000
13	,, C.	,,	0·004	—	4	400	No*
14	,, Da.	,,	0·004	—	4	900	,,
15	,, A.	,,	0·004	—	15	100	,,
16	,, Sa.	,,	0·004	—	4	100	,,
17	,, S.	,,	0·004	—	4	50	,,
18	,, B.	,,	0·004	130	4	50	,,
19	,, R.	,,	0·004	500	4	2,000	,,
20	,, Ri.	,,	0·004	700	8	2,000	,,
21	,, Bo.	,,	0·004	250	4	37	,,
22	,, Ba.	,,	0·03	10	1	1,100	,,
23	,, Bar.	,,	1·0	0·037	0·12	No*	,,
24	,, Ch.	,,	0·16	0·25	0·5	—	—
25	,, Cu.	,,	0·1	0·1	0·2	No*	No*

Abbreviations: SLE, systemic lupus erythematodes; nDNA, native DNA; dDNA, denatured DNA; fDNA, formalin-treated DNA; apurDNA, apurinic DNA; apyrDNA, apyrimidinic DNA.
* One serum unit is the minimal amount of serum containing antibodies capable of agglutinating 0·05 ml. of a 2·5 per cent suspension of DNA-sensibilized erythrocytes.
No*, not neutralized by dose of 1 mg.

systemic lupus erythematodes (sera 9 to 21). The fact that different amounts of denatured DNA are required to neutralize the antibodies from different sera suggests that the length of the region of the single-stranded molecule of DNA with which the antibodies of the second group can interact is less than the corresponding region of polynucleotide chain which will interact with antibodies of the first group. The third group is composed of those antibodies which are equally capable of interacting with native, denatured and formalin-treated DNAs (sera 22 to 25). These antibodies were found in patients suffering from a severe form of systemic lupus erythematodes (three of the patients died shortly afterwards). It should be noted that it is this type of antibody which is able to inhibit the activity of DNA as primer in the RNA polymerase reaction and furthermore influences DNA translating activity[8]. Prolonged storage of sera containing these antibodies results in a considerable reduction in their ability to interact with single-stranded and denatured DNA.

Recently, it was shown that the antinuclear factors present in the blood serum of different people may exhibit variable properties. Thus Bardawil *et al.*[9] have found that the antinuclear factor present in the blood serum of normal people interacts with deoxyribonucleoprotein at low ionic strengths only. On the other hand, the antinuclear factor from serum of patients with severe forms of systemic lupus erythematodes can react with deoxyribonucleoprotein at higher salt concentrations.

Data presented in Table 1 show that apurinic acid is also able to interact with antibodies, the amounts of this DNA required to neutralize the antibodies being variable and depending on the serum under test. These values are, however, always higher than that for the amount of single-stranded DNA necessary for the neutralization of the same quantity of antibody. This finding suggests that purines may be an important constituent of the sites of interaction in the DNA molecule. There is another explanation which is also plausible ; treatment of DNA with hydrochloric acid—used for the preparation of apyrinic DNA—is known to degrade DNA and it is possible that this may also lead to a significant degradation of the site of antibody interaction in the DNA molecule. In fact we found that the incubation of DNA with DNase considerably decreased the ability of the DNA to interact with antibodies.

In contrast with apurinic DNA, DNA lacking the pyrimidine bases is usually unable to interact with antibodies. Thus pyrimidine bases may be directly involved in the formation of the sites in the DNA molecule which will interact with the antibodies.

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Electrophoretic Determination of Albumin/Globulin Ratios in Mice

INFORMATION on the serum protein patterns of normal mice is necessary if these animals are to be used for studies in cellular immunity. However, except for Albritton's report[1], in which no indication was made of the animal's age, sex and strain, such published data are not available. This communication, therefore, provides some of this essential information; we have found that there are differences in serum protein patterns in different groups of mice, and that these patterns are related to age and sex.

All mice, with the exception of the pathogen-free ones, were outbred Swiss–Webster supplied by the University vivarium. They were housed at 22° ± 1° C under conditions calculated to minimize infection by microbes. They were fed a diet of 'Purina' rat chow and were given as much water as they wanted to drink. The following groups of mice were studied. (1) Normal: these were of known sex and age and at no time appeared ill or debilitated. (2) Immune: normal males, 10 weeks of age at the start of our injection schedule, received 1·0 mg intraperitoneally of bovine plasma albumin three times a week. They were bled 3 days after the seventh injection.

(3) Hyperimmune: normal males, 9 weeks old, received three subcutaneous injections of 2·0 mg of alum precipitated bovine serum albumin (BSA) which caused them to become immunologically paralysed. Once an anti-BSA haemagglutinin titre could be demonstrated, indicating an escape from paralysis, the animals were immediately bled. (4) Pathogen-free: 9-week-old males were obtained from the Charles River Mouse Farms. They had been maintained under aseptic conditions and were found to be serologically negative for common mouse viral and bacterial infections. They were bled immediately on receipt to ensure that their stipulated pathogen-free condition prevailed. (5) Newborn: 2-day-old suckling mice were bled and immediately killed (and hence were of undetermined sex). (6) Infected: normal 9-week-old females were injected intraperitoneally with approximately 1×10^7 coagulase positive *Staphylococcus aureus*.

All mice were bled from the retrobulbar plexus into sterile capillary tubes. Each serum was separated from the clotted blood by centrifugation and electrophoresed immediately, as poor reproducibility was obtained when frozen sera were used. A human control serum accompanied each series of test sera. In studies requiring frequent bleeding of the same mouse, we alternated plexus on successive bleedings.

All electrophoretic analyses were carried out with a microzone electrophoresis cell (Model *R*-101, Beckman Instruments, Inc.). The only changes from that procedure were: (1) that the time of electrophoresis was increased from 20 to 30 min, thus effecting better separation of serum proteins; and (2) that the *B*-2 buffer was changed after the second analysis rather than after the fourth, thus preventing precipitation of the buffer at the electrodes. When electrophoresis was completed, the cellulose acetate membranes were developed, dried, and scanned in a Beckman model *RB* 'Analytrol' with model *R*-102 'Microzone' scanning attachment.

Fig. 1 shows the wide range of albumin/globulin (A/G) ratios observed in the various groups of normal and experimental mice. The serum protein patterns of normal males differed greatly from those of females, as can be seen in Fig. 2. The male A/G ratios were not only less variable, but also displayed smaller standard deviations. The alterations of A/G ratios which occur temporarily in infected mice are shown in Fig. 3. Bacteriaemias persisted for 25–27 days, but blood samples analysed after 28 days were negative.

Our interpretation of these results is that the serum protein pattern of 13-week-old male mice remain more

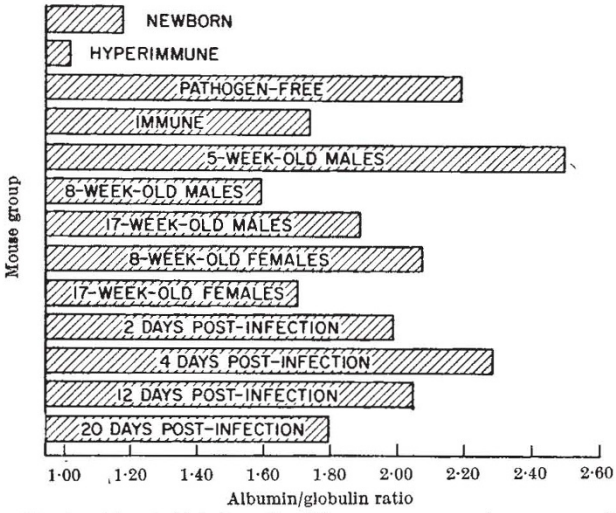

Fig. 1. Albumin/globulin ratio differences among various groups of mice. The infected animals were 8-week-old females at the time of infection. All groups except for the hyperimmune mice (four animals) contained at least ten animals.

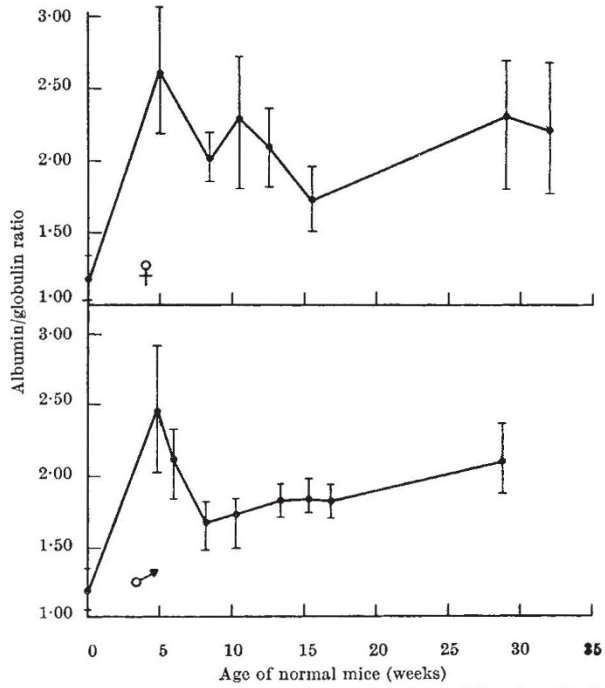

Fig. 2. Albumin/globulin ratios of normal males and females. Each point represents the mean value for at least ten mice. The line bisecting any given point represents the standard deviation for that age group.

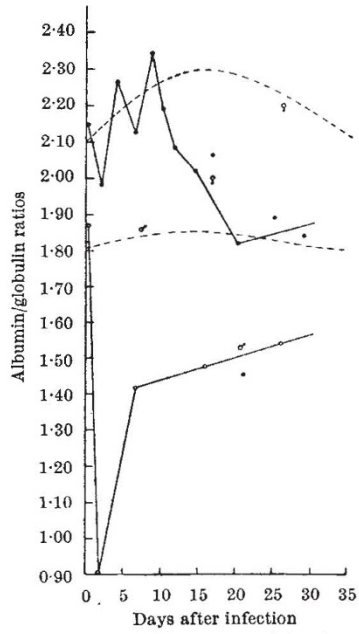

Fig. 3. Albumin/globulin ratios of 8-week-old females which were each injected intraperitoneally with 1×10^7 *S. aureus* compared with those of 13-week-old males which were similarly infected. The dotted lines represent the A/G ratios of uninfected animals.

stable and reproducible for at least 4 weeks than do those of other mouse groups. Serum protein patterns change dramatically with the age of the mice. The low A/G ratios found in newborn mice is attributable to colostrum-transferred maternal antibodies, and the very high A/G ratio found in 5-week-old mice denotes the immature state of their reticulo-endothelial systems. As the mice reach immunological maturity (at about 8 weeks of age) the A/G ratio reached a now, relatively stationary level. The fluctuation shown by the A/G ratios of normal females is believed to be related to oestrous cycle or other hormonal changes, or both. No attempt was made to

determine which group of animals elicits the highest antibody titre to any given antigen. It might well be that some group other than 13-week-old males should be used if the quantity of antibody produced is important. It is possible that the undulations in the A/G ratios exhibited by *S. aureus* infected females during the first 6–8 days after infection are caused by the focalization and subsequent breakdown of foci resulting in reinfection of the mice.

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Response to Insulin by Guinea-pig Taenia Coli

THE stimulating action of insulin on the metabolism of heart and striated muscle is well known, but the extent of its influence on smooth muscle is less clear and is variously assessed[1–8]. Preparations so far studied—such as aortic muscle[5–7] and intestinal segments[4]—suffer from the disadvantage that they are damaged during preparation or, like uterine muscle[1–3], have a highly individual endocrine response. Because of its ease of dissection and less specialized function, guinea-pig taenia coli muscle seemed an obvious tissue preparation with which to investigate the possible *in vitro* effects of insulin.

Insulin can without doubt enhance uptake of glucose by strips of this tissue *in vitro*, its retention of glucogen, the accumulation of the unutilized amino-acid, aminoisobutyrate, and the incorporation of carbon-14 labelled glycine into protein (Table 1). The figures given in Table 1, however, showing clear stimulating effects of insulin, are selected observations, and a large number of experiments were recorded in which a consistent stimulus by the hormone was not observed. The explanation for this is uncertain. For undeterminable reasons there seemed to be a much larger variation between strips from different animals than would be found with, for example, rat diaphragm muscle, and in different experiments the absolute values of glucose utilization and glycogen deposition moved between wider than usual limits. Axelsson, Bueding and Bülbring[9] have previously noted such fluctuations in the glycogen content, and they did not find an effect of insulin on glycogen retention. In most of the experiments in Table 1 the taenia preparations rested freely in a small quantity of incubation medium and consequently remained contracted. In other experiments the muscle strips have been tied to weights and placed under a tension of about 2 g. Under these conditions, the stimulating effect of insulin on the incorporation of ^{14}C-glycine was still observed (Table 1).

Table 1. EFFECT OF INSULIN ON THE METABOLIC ACTIVITY OF TAENIA COLI *in vitro*

	Effect of insulin: No insulin added	Insulin added (0·1 unit/ml.)	*P*
Unstretched tissue			
Uptake of glucose (mg/g tissue/h)	(6) 0·53 ± 0·08	0·95 ± 0·13	< 0·05
Glycogen content after incubation (mg glucose/g tissue)	(10) 0·78 ± 0·03	0·95 ± 0·06	< 0·05
Accumulation of carbon-14 labelled-aminoisobutyrate $\left(\frac{\text{(c.p.m./g tissue)}}{\text{(c.p.m./ml. medium)}}\right)$	(6) 5·3 ± 0·60	7·4 ± 0·69	< 0·05
Incorporation of carbon-14 labelled-glycine into protein (c.p.m./mg protein)	(9) 403 ± 28	632 ± 58	< 0·01
Tissue under tension			
Incorporation of carbon-14 labelled-glycine into protein (c.p.m./mg protein)	(6) 587 ± 51	657 ± 43	
	Difference 70 ± 19		< 0·02

Each figure is the mean ± *S.E.* of the mean of the number of observations in parentheses. For methods see refs. 23 and 24. For the experiments with unstretched tissue, two strips of taenia coli were removed from each guinea-pig (of either sex and of weight 200–400 g) and each strip cut into two approximately equal pieces. Medium consisted of Krebs-Ringer bicarbonate, gassed with 95 per cent O_2/5 per cent CO_2 and contained glucose (1 mg/ml.). Incubation was in 1 ml. for 2 h at 37° C with shaking. Carbon-14 labelled-glycine was added at a concentration of about 25 μM and 0·15 μc./ml. Carbon-14 labelled-aminoisobutyrate was added at a concentration of about 67 μM and 0·15 μc./ml. When the tissue was incubated under tension, the two ends of a single strip were tied together to a 5 g weight and the loop hooked around a stainless steel frame in 10 ml. of medium containing no glucose.

In a study of glucose metabolism by aortic muscle, Urratia, Beavan and Cahill[6] have also commented on the wide variability in behaviour between pieces of this tissue. They concluded that most of the apparent response to insulin of pieces of aortic muscle was due to the presence of adventitious tissue, and when the muscle was meticulously stripped, response to the hormone disappeared. Thus it is possible that with taenia some of the inter-animal variation results from varying degrees of contamination of the strips with adipose tissue, though it is debatable whether the amount adhering would be sufficient to account for all the insulin effects observed. On the other hand, guinea-pig adipose tissue is not very sensitive to insulin[10,11] and it is possible that the guinea-pig is just less sensitive to ox insulin than are some other species. Because the amino-acid sequence of guinea-pig insulin shows so many variations from other mammalian insulins[12], its use might be advantageous, although the available evidence[10] does not indicate differential sensitivity.

A point of contrast with heart or diaphragm muscle is the greater degree of concentration of aminoisobutyrate by taenia muscle than by these other tissues[13,14]. This may be related to the greater turnover of cellular sodium and potassium ions in smooth muscle as compared with striated muscle[15] and to the probable linkage of amino-acid uptake with the movements of ions[16]. Others have commented on the low oxidative metabolism of smooth muscle[5,6] and its high production of lactate. In our experiments, taenia produced an average of about 10 μmoles of lactate for each gram in a period of 2 h, which indicates the anaerobic glycolysis of most of the glucose taken up. The amount of lactate produced was not affected by insulin.

Various workers have questioned whether with smooth muscle there is as clear a distinction between intracellular and extracellular fluid as with striated muscle[15,17], and Goodford[18] has commented on variations in estimates of extracellular fluid in taenia according to the agents used for its determination. However, figures obtainable with sucrose and sorbitol (Table 2) agree well with Goodford's estimates with lithium and ethylsulphate, although they are higher than those found with inulin or polyglucose[19]. Differences between values found with inulin and other agents in various tissues are not unusual (Table 2). Yalcin and Winegrad[7] found a glucose space in aortic segments greater than the inulin and raffinose spaces and concluded that transport of glucose into the cell may not be the step limiting the rate of glucose utilization in that tissue. Any response to insulin under such circumstances would be of great interest. Our estimates, however, of the glucose space in taenia—24·9 ± 1·4 in the absence of insulin, and 27·5 ± 3·1 in its presence—do not suggest much intracellular accumulation. A preliminary report by Timms, Salama and Engstrom[20] suggests that the glucose space is increased by insulin.

Table 2. COMPARISON OF INULIN, SUCROSE AND SORBITOL SPACES IN VARIOUS MUSCLE PREPARATIONS

	Space (ml./100 g) measured with: Sorbitol	Sucrose	Inulin
Guinea-pig taenia coli	40·2	42·3	22·5
Rat intact diaphragm	24·5	23·8	23·5
Rat isolated hemidiaphragm	47·6	53·3	34·3
Rat extensor digitorum longus	35·8	34·4	19·2

Carbon-14 labelled sorbitol was added at a concentration of about 180 μM and 0·15 μc./ml. Inulin was added at a concentration of 0·6 per cent and sucrose at 1·25 per cent. All were extracted from the tissue after incubation by boiling and the sucrose and inulin estimated after hydrolysis to fructose (ref. 25).