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.

We thank Dr. Eli Sercarz for providing us with the hyperimmune mice used in these experiments. This investigation was supported by the National Science Foundation.

S. KLASKY
M. J. PICKETT

Department of Bacteriology,
University of California,
California.

[1] Albritton, E. C., *Standard Values in Blood* (W. B. Saunders Company, Philadelphia, 1951).

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).

There is thus some evidence that the taenia smooth muscle of the guinea-pig responds to insulin, though the quantitative significance seems to be variable. We may have to contend with the possibility that we cannot categorize all tissues as simply responsive or unresponsive to insulin, however, but that their response varies according to (as yet undetermined) conditions—a situation possibly akin to the limited response of brain slices[21,22].

We thank Dr. D. H. Jenkinson for much advice and discussion on the use and preparation of taenia coli.

S. H. GROSSMAN
K. L. MANCHESTER

Department of Biochemistry,
University College London,
Gower Street,
London, W.C.1.

[1] Krahl, M. E., *The Action of Insulin on Cells* (Academic Press, New York, 1961).
[2] Swigart, R. H., Goldberg, L. G., and Atkinson, W. B., *Endocrinology*, **68**, 643 (1961).
[3] Swigart, R. H., Wagner, C. E., Herbener, G. H., and Atkinson, W. B., *Endocrinology*, **70**, 600 (1962).
[4] Wool, I. G., and Manchester, K. L., *Nature*, **193**, 345 (1962).
[5] Urratia, G., Beavan, D. W., and Cahill, G. F., *Metabolism*, **11**, 530 (1962).
[6] Mulcahy, P. D., and Winegrad, A. I., *Amer. J. Physiol.*, **203**, 1038 (1962).
[7] Yalcin, S., and Winegrad, A. I., *Amer. J. Physiol.*, **205**, 1253 (1963).
[8] Lundholm, L., and Mohme-Lundholm, E., *Acta Physiol. Scand.*, **57**, 130 (1963).
[9] Axelsson, J., Bueding, E., and Bülbring, E., *J. Physiol.*, **156**, 357 (1961).
[10] Steinke, J., Miki, E., and Cahill, G. F., *New Engl. J. Med.*, **273**, 1464 (1965).
[11] Di Girolamo, M., and Rudman, D., *Amer. J. Physiol.*, **210**, 721 (1966).
[12] Smith, L. F., *Amer. J. Med.*, **40**, 662 (1966).
[13] Kipnis, D. M., and Noall, M. W., *Biochim. Biophys. Acta*, **28**, 226 (1958).
[14] Manchester, K. L., and Wool, I. G., *Biochem. J.*, **89**, 202 (1963).
[15] Burnstock, G., Holman, M. E., and Prosser, C. L., *Physiol. Rev.*, **43**, 482 (1963).
[16] Harris, E. J., and Manchester, K. L., *Biochem. J.*, **101**, 135 (1966).
[17] Hechter, O., and Halkerston, I. D. K., *The Hormones*, 5, 697 (Academic Press, New York, 1964).
[18] Goodford, P. J., *J. Physiol.*, **170**, 227 (1964).
[19] Goodford, P. J., and Hermansen, K., *J. Physiol.*, **158**, 426 (1961).
[20] Timms, A. R., Salama, A. E., and Engstrom, R. G., *Fed. Proc.*, **25**, 416 (1966).
[21] Rafaelsen, O. J., *Lancet*, ii, 941 (1958).
[22] R-Candela, J. L., and Martin-Hernandez, D., *Experientia*, **15**, 439 (1960).
[23] Manchester, K. L., and Young, F. G., *Biochem. J.*, **75**, 487 (1960).
[24] Manchester, K. L., *Biochem. J.*, **81**, 135 (1961).
[25] Pogell, B. M., *J. Biol. Chem.*, **211**, 143 (1954).

Epidermal Keratin and Epidermal Prekeratin

PROGRESS in understanding keratinization of the epidermis has been hampered by the difficulty of preparing a reproducible keratinous protein from the epidermal tissue[1,2]. Several laboratories[1-5] have obtained an alkali- or urea-extractable protein from human, mouse, and cow's snout epidermis which precipitates from solutions at *p*H 5·5. This protein is present in large amounts in fully keratinized human epidermal tissue (callus or stratum corneum)[1,2] and is believed to represent epidermal "keratin". Matoltsy[6-8] has isolated and characterized a similar protein from cow's snout epidermis which is soluble in acetic acid. This protein is termed "prekeratin" by virtue of its ready solubility from whole epidermis which includes incompletely keratinized cells (full-thickness cow's snout epidermis). This report compares human callus keratin (protein precipitating at *p*H 5·5) with the prekeratin of Matoltsy. Their similarities heighten optimism concerning the definition of an epidermal keratin sub-unit.

Human plantar callus keratin was obtained by alkaline solubilization[1,2] (100 ml. of 0·02 M sodium hydroxide (solvent) per gram of dried neutral-buffer washed ground callus, 22° C, 24 h) and by precipitation at *p*H 5·5 by the dropwise addition of dilute hydrochloric acid with *p*H monitoring. The protein was redissolved and precipitated four times.

The approximate molecular weight of protein dissolved out at *p*H 5·5 by 0·02 N sodium hydroxide was determined by comparing its mobility with that of proteins of known molecular weight utilizing 'Sephadex' thin-layer gel filtration, modified by Andrews[9]. 'Sephadex' (*G*-100 superfine, Pharmacia) was equilibrated for 72 h with 0·02 M sodium hydroxide, and plates of 0·5 mm thickness were prepared with a spreader (Shandon Unoplan). Cytochrome *C* (horse heart, type II, Sigma Chemicals), chymotrypsinogen (thrice crystallized, Worthington Biochemical Corporation), γ-globulin (human, fraction II, Sigma Chemical Company), and callus protein which had been precipitated four times and lyophilized at *p*H 5·5 was dissolved in 0·02 M sodium hydroxide and applied to the thin-layer plate (5 μl. aliquots representing 20 μg of protein). The solvent (0·02 M sodium hydroxide) was allowed to flow horizontally for appropriate periods of time and the proteins were located by exposure to iodine vapour. The reference proteins migrated as single compounds for a distance proportional to the log of their molecular weight (Fig. 1). The approximate molecular weight of the protein dissolved out at *p*H 5·5 was estimated at about 50,000 from its comparative migration.

Peptide fingerprints were prepared from tryptic digests of *p*H 5·5 protein (5 mg dissolved in 1·0 ml. of 0·1 M ammonium carbonate and incubated at 37° C for 24 h with 0·1 ml. of 1 : 100 trypsin crystallized three times, Worthington Biochemical Corporation). Two-dimensional peptide maps (descending chromatography of aliquots representing 1 mg of the original protein in a butanol–acetic acid–water medium (4 : 1 : 5); electrophoresis at 3,000 V for 1 h in a formic acid–acetic acid–water medium (6 : 24 : 170) *p*H 2·0) revealed forty-five or forty-six peptides with a light ninhydrin stain. This is comparable to the number of peptides identified by Rothberg[5] in tryptic digests of *p*H 5·5 protein obtained from human callus and psoriatic and ichthyotic scales. With the usual assumptions that complete digestion occurs and that the action of trypsin is specific to peptide bonds formed from arginine and lysine, the *p*H 5·5 protein used in this study should contain forty-four or forty-five arginine and lysine residues.

Amino-acid composition (duplicate hydrolysates, 6 N hydrochloric acid, 24 and 72 h at 110° C in tubes sealed under vacuum) of the four times precipitated *p*H 5·5 protein was determined by automated column chromatography ('Spinco' model 120 amino-acid analyser) and corrected for destruction or delayed release of specific residues[1,2]. Based on the amounts of arginine and lysine present in these samples, a molecular weight which would contain forty-four or forty-five arginine and lysine

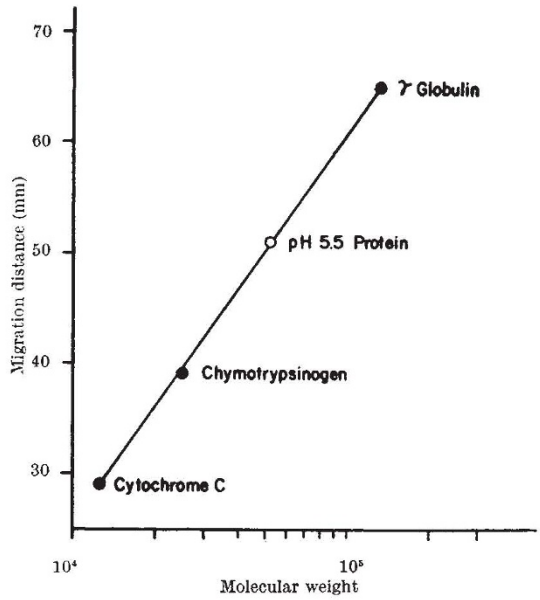

Fig. 1. Thin-layer gel filtration ('Sephadex *G*-100 Superfine' in 0·02 N sodium hydroxide at room temperature). Migration is plotted against (log) molecular weight (cytochrome *C*, 12,400; chymotrypsinogen, 25,000; γ-globulin, 160,000).