It can be seen from Fig. 2 that the variation in DNA distribution along the two chromosomes is quite striking although it should be stated that these are preliminary results and there is some reason to believe that the stain being used may in itself tend to be taken up with variable efficiency along the chromosome and therefore the stain density, which is of course the quantity being measured, is not a very accurate representation of the DNA distribution. This factor of the best choice of stain is being investigated at this laboratory, and it is hoped to find a stain which is sufficiently dark for scanning purposes and which also is taken up uniformly by the DNA present.

Other results on chromosomes 4 and 12 show essentially the same type of structure as indicated in Fig. 2, and a programme is now under way to discover the essential features of the DNA distribution along all the 46 normal human chromosomes.

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BIOCHEMISTRY

Secretion of Newly Synthesized Insulin *in vitro*

Up to the present, there has been little information available concerning the rate at which newly synthesized protein hormones may be secreted, following the stimulation of endocrine glands. In recent work on the synthesis of insulin within pancreas slices, *in vitro*, it became of interest to determine whether or not newly synthesized insulin may be released directly into the incubation medium, or alternatively whether or not release of insulin might be delayed following its accumulation within β-granules. It has already been suggested by Lacy[1] that release of insulin from β-cells in response to glucose involves the movement of these granules to the cell surface, where they are released into blood vessels.

In the experiments to be reported, insulin was labelled by incorporation of label into pancreas slices *in vitro*, and its release into media containing glucose studied. When rabbit pancreas slices are incubated in physiological media, such as Krebs–Ringer bicarbonate, there is a small release of insulin at low glucose concentrations, though this is very greatly increased when the sugar concentration is raised. The increased release of insulin has been shown to reflect, at least qualitatively, the physiological behaviour of β-cells *in vivo*[2]. To label the insulin, rabbit pancreas slices were incubated for 1 h with tritiated leucine of high specific activity by methods which have already been described in detail elsewhere[3,4]. The incubation medium was then poured off and the pancreas slices washed thoroughly with fresh medium to remove excess radioactivity. The slices were then incubated for 30 min in a medium containing low concentrations of glucose (50 mg/100 ml.). For the final 1-h incubation phase, the medium was again discarded and the pancreas slices divided into two parts, one being incubated in a medium of low glucose concentration (50 mg/100 ml.), the other being incubated in medium containing a much higher concentration of glucose (250 mg/100 ml.). The medium was gassed with 95 per cent oxygen and 5 per cent carbon dioxide before each incubation phase. Insulin in the medium derived from the final incubation phase was assayed by an immunological method[5], and isolated from the medium so that its specific radioactivity might be determined.

The isolation of labelled insulin in the medium was carried out by an immunological technique, based on the precipitation of an insulin–antibody complex by means of an anti-γ-globulin. For this purpose, the whole medium was dialysed against distilled water and freeze dried. The proteins were then dissolved in a small volume of 0·003 M hydrochloric acid, and allowed to react with 0·5 ml. of a potent anti-serum made by repeated injection of crystalline ox insulin into guinea-pigs[6]. The whole γ-globulin fraction was then precipitated by the addition of a rabbit–anti-guinea-pig γ-globulin serum. The precipitate obtained was spun off, and after washing with saline, the insulin was regenerated from it by acid, and further purified by two-dimensional chromatography and electrophoresis on paper as previously described[4]. Insulin regenerated was assayed in the extract by immunoassay. The results of two representative incubations are shown in Table 1.

Table 1

Exp.	Insulin release into medium in final incubation phase (mU/flask/h)		Specific radioactivity of insulin extracted from medium (c.p.m./μg)	
	Low glucose	High glucose	Low glucose	High glucose
1	6·48	24·4	1,040	567
2	7·36	26·1	912	552

It will be seen that when pancreas slices are incubated in a medium containing a low concentration of glucose for 1·5 to 2·5 h after the start of labelling, there is some release of labelled insulin. However, when the final phase of incubation is in a medium of high glucose concentration, insulin release from the slices is increased several-fold while the specific activity of the insulin falls markedly. Very recently, similar results have been obtained from experiments in which the medium was extracted with acidic ethanol and the crude insulin precipitated with ether-ethanol[7] before purification by the method already described here. In two such experiments the ratio of insulin output in the high glucose medium to that in medium of low glucose content was 4·08 : 1, while the ratio of specific activities of the insulin extracted was 0·35 : 1. It seems, therefore, that the greater part of the insulin secreted in response to a glucose stimulus has not been synthesized in the preceding 1·5–2·5 h. These results are in marked contrast with those obtained for the secretion from liver of newly synthesized albumin which is known not to be stored in granules[8].

In pancreatic acinar tissue there is evidence from autoradiographic studies that newly synthesized secretory proteins appear first in the rough surfaced elements of the endoplasmic reticulum, then in the Golgi complex and finally after 1 h in the zymogen granules, from which they begin to be discharged 2 h after synthesis[9]. From the results described here, it seems possible there may be analogies between the secretion of newly synthesized insulin and the discharge of recently made secretory proteins.

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