(1966) of a marked increase in kidney weight and an increase in mitoses of tubular cells.

The mortalities in the animals receiving HgCl_2 are shown in Table 2.

TABLE 2
 HgCl_2 injected animals

	Folic acid treated groups			Control groups given dextrose or NaHCO_3		
	No. of animals	Survived	Died	No. of animals	Survived	Died
I	8	4	4	4	2	2
II	9	4	5	10	5	5
III	10	2	8	8	4	4

- I. F.A. or dextrose injected 48 hr after HgCl_2 poisoning.
 II. F.A. or dextrose injected 24 hr after HgCl_2 poisoning.
 III. F.A. or NaHCO_3 injected 24 hr after HgCl_2 poisoning.

On summed figures for Control and Treated Groups

$$\chi^2 = 0.39 \text{ for 1 d.f.}$$

$$0.7 > P > 0.5.$$

It will be seen that approximately 50% of animals died and that there was no significant difference between those receiving folic acid and a control solution of either dextrose or NaHCO_3 .

The kidney weights of animals examined are shown in Table 3. (The numbers of animals dying on days other than the fourth were so small as to make comparison of kidney weights difficult. Therefore weights derived from deaths on day 4 only are shown in the table.)

Although the mean weights of the kidneys of animals receiving folic acid and mercury were greater than those receiving a control solution and mercury, the differences were not significant.

Moreover, histological examination did not reveal any difference between these groups in respect of mitotic activity or appearance of the tubule cells.

TABLE 3
Mean kidney weights of rats (g)

		Died on 4th day	Survived 14 days
Non-treated normal kidney	1.301		
Folic acid injected kidney	2.276		
HgCl_2 poisoned kidney		2.361	1.861
HgCl_2 poisoned + folic acid treated kidney		2.915	2.004

Discussion

This study confirms the findings of Taylor *et al.* (1966) and Haddow (1954) that folic acid causes an increase in kidney weight and a multiplication of tubule cells in normal animals. However, when it was administered to rats poisoned with mercury, no such effect could be demonstrated and, moreover, the survival of animals so treated was unaffected.

References

- HADDOW, A. (1954) *Chemistry and Biology of Pteridines: Ciba Foundation Symposium*, p. 100. Churchill, London.
 NOLTENIUS, H., SCHELLHAS, H. & OEHLERT, W. (1963) Histoautoradiographische Untersuchungen mit ^3H -Thymin der Tubuluszellregeneration nach akuter Sublimatvergiftung von Ratten. *Beitr. path. Anat.* **129**, 90.
 OLIVER, J. (1954) The structural and functional aspects of recovery from acute renal failure. *The Kidney: Ciba Foundation Symposium*, pp. 1-14. Churchill, London.
 TAYLOR, D.M., THRELFALL, G. & BUCK, A.T. (1966) Stimulation of renal growth in the rat by folic acid. *Nature (Lond.)*, **212**, 472.

Synalbumin insulin antagonism

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USING the technique of the isolated rat diaphragm to measure insulin activity and antagonism, our initial studies on untreated or uncontrolled insulin-requiring diabetic patients showed that their plasma inhibited the activity of insulin *in vitro* (Vallance-Owen, Hurlock & Please, 1955). It was then found that this antagonism to insulin was associated with the

albumin fraction of the plasma proteins (Vallance-Owen, Dennes & Campbell, 1958a). Whole plasma from normal subjects and from obese maturity-onset diabetics, who ordinarily do not require insulin therapy, has no measurable insulin antagonism. Nevertheless, when normal plasma is broken down into its various constituents, insulin antagonism can be detected in the

albumin fraction, although it is less active than the corresponding fraction prepared from the plasma of the insulin-requiring diabetics.

When tested at 3.5–5.5%, both diabetic and normal albumin completely inhibited the effect of 1000 micro-units/ml of insulin added *in vitro*. At 1.25%, however, the diabetic albumin was still highly antagonistic, whereas albumin from normal subjects was inactive.

This antagonistic activity appears to depend upon both the pituitary gland and the adrenal steroids (Vallance-Owen, Dennes & Campbell, 1958b; Vallance-Owen & Lilley, 1961); it is not due to the albumin itself but to some substance associated with it, hence the term synalbumin antagonist.

It has also been found that albumin from obese maturity-onset diabetics and from latent diabetics is antagonistic to insulin when tested at 1.25% to the same extent as albumin prepared from insulin-requiring diabetic patients, and to a considerably greater degree than normal albumin, which is inactive at this concentration. Moreover, patients with the diabetic syndrome of carbohydrate intolerance, but suffering from definite pancreatic disease such as acute pancreatitis or haemochromatosis or who have sustained total pancreatectomy, have no increased antagonism to insulin associated with their plasma albumin (Vallance-Owen, 1962, 1964).

Thus essential diabetics, whether insulin-requiring, obese or in the latent phase, have more synalbumin antagonism than normal subjects or pancreatic diabetics. These observations indicate that excessive synalbumin antagonism (synalbumin-positive) can be regarded as a biochemical marker to ascertain whether or not a given individual is constituted as an essential diabetic—without reference to carbohydrate intolerance. On this premise the presumed genetic transmission of essential diabetics has been examined by studying the relatives of patients suffering from this condition.

The results strongly suggest that excessive synalbumin antagonism is inherited as an autosomal 'dominant' character. Ninety-seven members of nine families were studied, thirty-nine were synalbumin-negative whereas fifty-eight were synalbumin-positive, but only sixteen of these latter have overt carbohydrate intolerance. A further three have spontaneous hypoglycaemia whereas the remainder are quite asymptomatic. These observations suggest that overt carbohydrate intolerance is relatively uncommon or will be a late event in many people constituted as essential diabetics (Vallance-Owen, 1966).

Recently a number of studies have been made on the albumin prepared from cord blood of synalbumin-negative mothers and of synalbumin-positive mothers, who were either in the latent or overt phase of diabetes. Twenty-seven observations were made and the results are shown in Table 1.

TABLE 1

Mothers	Cord blood	
	Positive	Negative
15 +	9	6
12 –	3*	9

Synalbumin-positive = antagonistic to insulin at 1.25%. Synalbumin-negative = non-antagonistic to insulin at 1.25%.

* Two fathers +, one not tested.

It can be seen that synalbumin-positive mothers have cord bloods which are negative as well as positive. Therefore, it is clear that a child at birth can either be synalbumin-positive (constituted as a diabetic) or synalbumin-negative (normal).

An aetiological concept of diabetes mellitus

These observations indicate that a fundamental abnormality in essential or idiopathic diabetes mellitus is increased synalbumin antagonism to insulin—an exaggeration of the normal—which is present from birth and is apparently inherited by essential diabetics as an autosomal 'dominant' character. The clinical types of diabetes, and the time of onset of carbohydrate intolerance, will then depend on the degree of antagonism—modified by environmental factors—and on the ability of the beta-cells of the pancreas to withstand the challenge. There will be a normal variation in the resilience of these cells. It may also be important, with respect to development of overt diabetic intolerance, whether the diabetic constitution is inherited through the mother or through the father: that is, is the child more or less likely to develop carbohydrate intolerance in later life if the islets are hypertrophied *in utero*, as is well known to be the case when the affected parent is the mother?

The synalbumin antagonism is apparently dependent upon the pituitary–adrenal system. Therefore, the already increased antagonism will be even further increased under certain physiological and environmental conditions—notably the growth spurt, infection, the menopause, mental stress for any reason, or when adrenal

corticosteroids are administered. These are times and situations which are well known to precipitate carbohydrate intolerance in some susceptible individuals or to aggravate this condition if it already exists. In this connection as our preliminary studies indicate that approximately 25% of the population are constituted as diabetics, it is of interest that only about 25% of patients suffering from acute acromegaly or Cushing's syndrome actually develop overt diabetes. It is suggested that only those individuals constituted as diabetics exhibit carbohydrate intolerance when they develop the above conditions.

Whatever the environmental reason, in a certain number of constituted diabetics the beta-cells of the pancreas ultimately begin to fail; then insulin production falls short of requirements to a greater or lesser degree, leading to the well-recognized groups of insulin-requiring and obese diabetic patients, respectively. Nevertheless, the phase before carbohydrate intolerance supervenes is certainly more interesting and probably more important, especially as the abnormality is only a late or very late symptom of a condition present from birth.

Nature of the synalbumin antagonist

The synalbumin antagonist is unlikely to be a free lipid, fatty acid or steroid-type compound and is possibly a polypeptide (Vallance-Owen & Lilley, 1961). Moreover, its molecular weight is likely to be 4000 or less.

There is an enzyme, mainly concentrated in the liver, which has a major specificity for the degradation of the insulin molecule. This enzyme, previously known as insulinase (Mirsky & Broh-Kahn, 1949), has now been isolated and characterized (Tomizawa & Halsey, 1959). The purified enzyme, now called glutathione insulin transhydrogenase, catalyses the reductive cleavage of the disulphide bonds of insulin by glutathione, resulting in the formation of the 'A' and 'B' chains of the insulin molecule. The 'B' chain is a stable polypeptide of 3800 molecular weight with two -SH groups per molecule, which in its reduced state is virtually insoluble in physiological buffers.

Radioactive insulin in the presence of the purified enzyme glutathione and serum is cleaved into two radioactive components. These compounds, distinguished from ^{131}I -insulin at the origin by their electrophoretic migration in the α_2 -globulin and albumin regions, have been isolated and identified as the radioactive 'A' and 'B' chains respectively. It has also been established that the 'B' chain migrating with the albumin

electrophoretically is, in fact, bound to albumin (Ensinnck *et al.*, 1964).

Inhibitors of biochemical reactions are frequently similar to the metabolites which stimulate the reaction: thus the 'B' chain might be capable of inhibiting the action of its parent molecule and being associated with albumin might be equated with the synalbumin insulin antagonist.

It has now been found that when the albumin 'B' chain complex resulting from incubation of small amounts of 'B' chain with non-antagonistic albumin is assayed in the rat diaphragm system with insulin, marked antagonism to the hormone occurs (Ensinnck, Mahler & Vallance-Owen, 1965). Studies which are continuing also indicate that the synalbumin antagonist and the 'B' chain of insulin have a number of physiochemical similarities, notably molecular weight, the type of bonding to albumin, and ionic charge as well as biological activity. We have previously shown that the heat-coagulated filtrate from antagonistic albumin prepared either from diabetic or normal subjects is also antagonistic to insulin (Vallance-Owen & Lilley, 1961), and also antagonistic albumin can be rendered non-antagonistic in several different ways.

Recently some preliminary immunological studies have been carried out. We have attempted to make antibodies in rabbits to the heat-coagulated filtrate (supernatant) from diabetic albumin and non-antagonistic albumin.

The heat-coagulated material dissolved in saline, with the addition of Freund's adjuvant (complete), was injected subcutaneously into the rabbits each week for 8 weeks. After this time the animals were bled and serum obtained, from which globulin was prepared using DEAE-cellulose and diluting with phosphate buffer.

The γ -globulin from rabbits treated with non-antagonistic material did not affect the inhibiting properties of the heat-coagulated filtrate from normal albumin or from 'B' albumin—the antagonism is of the same order as when these preparations are tested alone.

However, with γ -globulin from the rabbits injected with heat-coagulated filtrate from diabetic albumin, the inhibiting effect is abolished not only from the filtrate of diabetic but also from the filtrate of normal albumin and 'B' albumin.

These preliminary observations, which are in process of being repeated, further suggest that the synalbumin antagonist and the 'B' chain of insulin are identical.

Further studies

The cause of the increased synalbumin antag-

onism of constituted diabetics is quite unknown. However, if it can finally be proved that the synalbumin antagonist is in fact the 'B' chain of insulin, then the basic abnormality of essential diabetics which results in excessive circulating 'B' chain bound to albumin needs to be elucidated. Several possibilities exist:

(1) Decreased breakdown in the periphery of the albumin-bound 'B' chain.

(2) Increased 'B' chain production in the pancreas as a result of some abnormality of insulin production.

(3) Alteration in the hepatic or peripheral degradation of insulin, resulting in excess 'B' chain available for binding to albumin.

(4) A subtle change in the structure of diabetic albumin allowing it to carry larger quantities of 'B' chain.

References

- ENSINCK, J.W., COOMBS, G.J., WILLIAMS, R.H. & VALLANCE-OWEN, J. (1964) Studies *in vitro* of the transport of the A and B chains of insulin in serum. *J. biol. Chem.* **239**, 3377.
- ENSINCK, J.W., MAHLER, R.J. & VALLANCE-OWEN, J. (1965) Antagonism of insulin action on muscle by the albumin-bound B-chain of insulin. *Biochem. J.* **94**, 150.
- MIRSKY, I.A. & BROH-KAHN, R.H. (1949) The inactivation of insulin by tissue extracts. I. The distribution and properties of insulin inactivating extracts (Insulinase). *Arch. Biochem.* **20**, 1.
- TOMIZAWA, H.H. & HALSEY, Y.D. (1959) Isolation of an insulin-degrading enzyme from beef liver. *J. biol. Chem.* **234**, 307.
- VALLANCE-OWEN, J. (1962) Diabetes mellitus—Causation. *Proc. roy. Soc. Med.* **55**, 207.
- VALLANCE-OWEN, J. (1964) Synalbumin insulin antagonism and diabetes. *Ciba Fdn Colloq. Endocr.* **15**, 217.
- VALLANCE-OWEN, J. (1966) The inheritance of essential diabetes mellitus from studies of the synalbumin insulin antagonist. *Diabetologia*, **2**, 248.
- VALLANCE-OWEN, J., DENNES, E. & CAMPBELL, P.N. (1958a) Insulin antagonism in plasma of diabetic patients and normal subjects. *Lancet*, **ii**, 336.
- VALLANCE-OWEN, J., DENNES, E. & CAMPBELL, P.N. (1958b) The nature of the insulin antagonist associated with plasma albumin. *Lancet*, **ii**, 696.
- VALLANCE-OWEN, J., HURLOCK, B. & PLEASE, N.W. (1955) Plasma insulin activity in diabetes mellitus measured by the rat diaphragm technique. *Lancet*, **ii**, 583.
- VALLANCE-OWEN, J. & LILLEY, M.D. (1961) An insulin antagonist associated with plasma-albumin. *Lancet*, **i**, 804.

Regional differences in the lung

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IN OCTOBER 1957, Dr M. Ter-Pogossian of St Louis, U.S.A., visited Hammersmith Hospital and demonstrated the use of a new radioisotope for biological research, oxygen-15, half-life 2 min. Air was blown through the beam of the M.R.C. cyclotron and collected in a bag. Dr Ter-Pogossian took a breath of this: a counter at his finger-tips registered radioactivity within seconds. At the subsequent colloquium arranged by the M.R.C. Radiotherapeutic Research Unit, Professor McMichael was enthusiastic about the potential of this new tool and the active research programme using radioactive gases which has developed owes much to his support.

It was soon realized that marked differences in blood flow and ventilation as measured with radioactive oxygen exist between the top and bottom of the lung. At this time, very little was known about topographical differences within the lung; a well-known textbook of respiratory physiology devoted three lines to the subject! However, in the last 10 years it has been shown

that there are great regional differences of blood flow, ventilation, gas exchange and morphology in the lung, and that these frequently have important clinical implications. In this short article it is only possible to give a brief summary of some of these advances.

Blood flow

When a subject inhales radioactive oxygen and holds his breath, the rate of removal of radioactivity from any region of the lung as measured with external counters is a measure of the local blood flow. In practice, oxygen-labelled carbon dioxide is a more useful gas because its removal by the blood flow is faster. The same information can be obtained using the reactor-produced radioactive gas xenon-133 and since this has a half-life of 5 days, it is a more convenient technique and is in general use throughout the world.

In the normal upright lung, blood flow is found to decrease rapidly from the bottom to the top reaching low values at the apex (Fig. 1)