Most soft exudates become fluorescent, and in some the fluorescence precedes the visible exudate and persists after it has disappeared.

We would like to express our thanks to Professor J. McMichael, F.R.S., for his interest and encouragement, and to Professor N. Ashton, of the Institute of Ophthalmology, for valuable suggestions and criticism.

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SOME BIOCHEMICAL CHANGES IN THE TRANSPLANTED KIDNEY

A PRELIMINARY REPORT

BY

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Homotransplanted kidneys of the dog are known to survive for several days before their function is abruptly terminated by the onset of an irreversible anuria. Possible causes of this anuria were discussed but no satisfactory explanation was put forward (Dempster, 1955), although at this time a reaction of the kidney involving a tubular metabolic upset was thought possible. Homotransplanted kidneys in general show little histological alteration from the normal apart from some interstitial oedema and a plasma-cell infiltration. Recent investigations into the source of the cellular infiltration (Fowler and West, 1960, 1961; Porter and Calne, 1960; Dempster and Williams) indicate that a variable number of these cells originate in the host and may be implicated in the rejection process.

The available data on the physiological performance of homotransplanted kidneys (Dempster, 1953a; Fedor *et al.*, 1959; Tyler *et al.*, 1962) give little indication of any pathological processes which could explain the sudden anuria.

In an attempt to elucidate further the sudden terminal events, a series of biochemical studies have been undertaken on transplanted kidneys. The respiratory enzymes succinic dehydrogenase and malic dehydrogenase and the glycosidic enzymes β-glucuronidase, β-galactosidase, and β-glucosaminidase have been measured quantitatively in normal, autotransplanted, and homotransplanted kidneys. Preliminary studies suggested that transplants possessed much lower enzyme levels than normal kidneys. Factors such as the interstitial oedema and cellular infiltration, which may give rise to apparent changes in kidney enzyme levels, have been assessed by extending the preliminary studies to include enzyme histochemistry and microchemical analyses. Enzymes studied histochemically include succinic and malic dehydrogenases, D.P.N.H. (reduced diphosphopyridine nucleotide) diaphorase, cytochrome oxidase, acid phosphatase, and β-glucosaminidase.

Chemical analyses included D.N.A. (desoxyribose nucleic acid), R.N.A. (ribonucleic acid), protein, and methionine. Hydroxyproline and hexosamine (representing collagen and mucopolysaccharide respectively), and the wet weight to dry weight ratio of the tissue were also estimated.

Materials and Methods

Animals.—All dogs used in these experiments were greyhound bitches with an average weight of about 22 kg.

Surgical Techniques.—Kidneys were transplanted by techniques previously described (Dempster, 1953a, 1954).

Removal of Kidneys.—Functioning autotransplants were removed between the second and seventh days after transplantation. Homotransplants have been divided into two main groups—those which were removed between the second and fourth days while still functioning, and those which proceeded to oliguria. Oliguric kidneys have been further divided into two subgroups according to the day that they became oliguric. The first subgroup includes those kidneys becoming oliguric on the third, fourth, fifth, or sixth day; the second subgroup includes kidneys becoming oliguric later than the sixth day. The longest survival was 17 days.

Assessment of Oliguria.—Kidneys were adjudged to be truly oliguric when a sudden decrease in daily urine output occurred together with a poor response to successive infusions of 500 ml. of 0.9% saline and 300 ml. of 2% saline. This condition was often accompanied by a high blood-urea level and a toxic syndrome (Dempster, 1953b).

Enzymology.—β-glucuronidase, β-galactosidase, and β-glucosaminidase were estimated by hydrolysis of phenolphthalein glucuronide, *o*-nitrophenyl-β-D-galactoside and *p*-nitrophenyl-β-D-glucosaminide respectively (Findlay *et al.*, 1958; Levvy and Marsh, 1959; Conchie *et al.*, 1959). Malic and succinic dehydrogenases were estimated manometrically (Umbreit *et al.*, 1957). All enzymes were estimated on complete homogenates of kidney cortex, the media for homogenization being water for the hydrolases and 0.25*M* sucrose for the dehydrogenases.

Histochemistry.—Small blocks of kidney were frozen in liquid oxygen and sections cut at 6 microns in a Bright cryostat at −15° C. Succinic dehydrogenase, malic dehydrogenase, and D.P.N.H. diaphorase were demonstrated by the M.T.T. method of Pearse (1960), cytochrome oxidase by the method of Burstone (1960), and acid phosphatase by the method of Burstone (1958). β-Glucosaminidase was demonstrated using naphthol AS-LC-N-acetyl-β-glucosaminide as substrate (kindly supplied by Dr. D. Janigan).

Histology.—Material for paraffin sections was taken immediately on removal of the kidney and fixed in 10% formol-saline. Sections were stained with haematoxylin and eosin or methyl-green-pyronine.

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Chemical Analysis.—All analyses were carried out on samples which had been dialysed against distilled water and dried at 60° C. *in vacuo* over P_2O_5. Protein was assayed by the method of Lowry *et al.* (1951). Methionine was bioassayed using a methionine requiring auxotroph of *Escherichia coli*. Maximum growth was obtained on homogenates digested in 5*N* HCl at 110° C. for five hours. Hexosamine was estimated by the method of Rondle and Morgan (1955). Hydroxyproline was measured colorimetrically using a micromodification of the method of Fels (1958): homogenate (30 mg.) was digested in 6*N* HCl at 140° C. in a sealed ampoule for five hours. After neutralization the digest was passed through a column of activated charcoal (300 mg.) and made up to 10 ml. One ml. was taken for colorimetry. D.N.A. and R.N.A. were assayed by the Schmidt-Thannhauser-Schneider procedure, as described by Volkin and Cohn (1954) using calf thymus D.N.A. and D-ribose, respectively, as standards. The diphenylamine reaction (Dische, 1955) was used for D.N.A. and the orcinol reaction (Dische and Schwarz, 1937) for R.N.A. estimations respectively.

Results

Enzymology

The Table shows the results of enzyme estimations on normal, autotransplanted, and homotransplanted kidneys. Activities were referred to dialysed dry weight, and activities of the transplants are expressed as percentages of the normal value.

Mean Enzyme Levels of Normal and Transplanted Kidneys. Values are Expressed as Percentage of the Activity Shown by Normal Kidneys

Enzyme	Normal	Functioning Autotransplants	Functioning Homotransplants, 2–4 Days	Oliguric Homotransplants	
				3–6 Days	7–17 Days
Malic dehydrogenase	100 (82·1±22·5)	82·6	55·0	63·2	46·3
Succinic dehydrogenase	100 (69·0±20·5)	75·4	51·6	57·8	44·7
β-Glucuronidase	100 (11·4±1·5)	87·7	72·8	75·4	121·0
β-Galactosidase	100 (11·5±2·0)	91·3	73·9	79·1	141·0
β-Glucosaminidase	100 (340±36)	82·6	67·9	Insufficient data	116·0
No. of kidneys examined	15	10	8	8	4

Figures in parentheses are the actual enzyme units with standard deviation. Dehydrogenases—μl. O_2 absorbed/mg./hr. Glycosidases—μg. aglycone released/mg./hr.

Autotransplants.—Each enzyme had a reduced activity, between 75% (succinic dehydrogenase) and 91% (β-galactosidase) of normal. The differences shown by the two dehydrogenase and β-glucuronidase activities were significantly lower than normal ($P<0.05$).

Homotransplants.—(*a*) *Functioning kidneys:* The mean values of each enzyme showed further decreases in activity. These further decreases were more pronounced in the dehydrogenases, these activities being significantly lower than in the autotransplants ($P<0.05$). (*b*) *Oliguric (3rd–6th day) kidneys:* Although the means indicate a slight rise when compared with the previous group, the differences were not significant. Insufficient data were available to assess the mean β-glucosaminidase activity in this group. (*c*) *Oliguric (7th–17th day) kidneys:* All the kidneys in this small group showed striking increases in glycosidase activities to more than 100% of the normal values; dehydrogenases, on the other hand, showed further decreases.

Histochemistry

Autotransplants.—(1) *Renal tubules:* Differences between autotransplants and normal kidneys were small. Small decreases in the level of all enzymes were observed, especially in the proximal convoluted tubules. Occasional proximal convolutions showed pronounced damage and loss of enzyme activity. This destruction appeared to be focal. (2) *Infiltrating cells:* None were observed.

Homotransplants: Functioning Kidneys.—(1) *Renal tubules:* Small changes similar to those occurring in autotransplants were observed. No widespread damage was apparent. Acid phosphatase was present in small discrete granules. (2) *Infiltrating cells:* These were few. Acid phosphatase and β-glucosaminidase were present. Of the four respiratory enzymes examined only malic dehydrogenase showed any activity in these cells. This reaction was faint.

Homotransplants: Oliguric Kidneys.—(1) *Renal tubules:* Pronounced reductions in enzyme levels were observed, all enzymes being affected. Tubule cells were flattened and the tubules separated. Acid phosphatase reactions were strikingly different from those in autotransplants. The reaction products were clumped in large masses, often around the nucleus. (2) *Infiltrating cells:* These were present in large numbers. Staining for acid phosphatase and β-glucosaminidase was pronounced. No respiratory enzyme other than malic dehydrogenase was observed, and this in small quantity. Homotransplants that had survived for periods of seven days or more showed a very heavy cellular infiltration, all cells showing acid phosphatase and β-glucosaminidase activity. Serial sections stained with methyl-green-pyronine showed many of these cells to be of the plasma-cell series.

Chemical Analyses

Analyses were carried out on 15 normal kidneys, 9 autotransplants, 8 secreting homotransplants, and 9 oliguric homotransplants.

Autotransplants.—The results indicate that the oedema fluid contributes insufficient non-dialysable material to account for the observed decreases in enzyme levels. Increases in D.N.A. and R.N.A. percentages were found, although the additional analytical data do not offer an obvious explanation.

Homotransplants.—(*a*) *Functioning* (2nd–4th day): In addition to the oedema fluid the effect of the cellular infiltration must be considered. The cellular infiltration at this stage is minimal (Dempster, 1955), and, apart from a slight increase in R.N.A. percentage, the analytical results are similar to those obtained from autotransplants. Thus the contribution of the oedema fluid and the plasma cells to the dry weight of the kidneys can be regarded as negligible. (*b*) *Oliguric* (3rd–17th day): Those kidneys of this group which survived longer than six days had an extremely heavy plasma-cell infiltration. The mean value for the D.N.A. percentage is more than twice the normal value and 60% higher than that of secreting homotransplants. The mean collagen value is depressed to about 75% of the normal. The contribution of the cellular infiltration to the dry weight of the kidney is therefore probably considerable in kidneys surviving more than six days. The mean dry weight : wet weight ratios of autotransplants and secreting and oliguric homotransplants showed a progressive decrease in this order. The oliguric homotransplants have a ratio 72% of normal.

Discussion

Preliminary experiments indicated that the enzyme levels of transplanted kidneys were lower than normal when expressed in terms of dry weight. In order to check the accuracy of the observed enzyme levels any contribution to the dry weight by the oedema fluid and the cellular infiltration (in the case of homotransplants) was assessed by chemical analysis. Such analysis indicated that the falls in enzyme levels are real, and that only in the long-surviving homotransplants are the enzyme levels seriously affected by the cellular infiltration. Dry weight appears to be a satisfactory parameter on which to base the enzyme activities of kidneys removed within six days of homotransplantation. Wet weight is not a satisfactory parameter.

The lower enzyme levels observed in autotransplants by quantitative estimation have been supported by the histochemical results. These have indicated a small overall decrease in all of the enzymes studied, with pronounced damage and loss of enzyme activity in occasional proximal convolutions. The loss in enzyme activity is presumably due to the surgical procedure and the period of ischaemia (20–30 minutes). Similar enzyme levels might be expected in functioning homotransplants, but these showed a further fall in enzyme levels, particularly in the case of dehydrogenases. The reason for these further and non-uniform decreases is at present obscure, especially as the histochemistry of these kidneys is similar to that of the autotransplants. None of the enzymes studied quantitatively showed any change in level at the oliguric stage. The only difference observed between functioning and oliguric homotransplants, apart from the degree of cellular infiltration, was a striking intracellular redistribution of acid phosphatase, demonstrated histochemically. The alteration in the localization of this enzyme is not a specific characteristic of a rejecting homotransplanted kidney, but is an early indication of cell damage (Novikoff, 1959; Becker and Barron, 1961; Janigan and Santamaria, 1961). The quantitative changes in enzyme levels observed in the longer-surviving homotransplants can be explained by the massive cellular infiltration.

The results presented here provide no evidence of a sudden metabolic shutdown occurring at the oliguric stage (Tyler *et al.*, 1962), nor is there evidence for a gradual decline in metabolism during the survival of a homotransplant. The indications are that the low enzyme levels characteristic of the homotransplant occur within the first few days and thereafter remain almost constant. If this interpretation is correct it implies that an early, perhaps immediate, reaction occurs within the kidney. This reaction is not necessarily a decisive factor in the final process of transplant destruction.

The observations of Chiba *et al.* (1962) on the metabolism of the transplanted heart suggested that the rejection process might be a continuous one. These workers were unable to assess any changes in metabolism due to surgery *per se*, and failed to take into account any changes due to the infiltrating cells. These workers suggested that aerobic glycolysis was taking place with a conversion of pyruvate to fatty acid rather than pyruvate oxidation via the Krebs tricarboxylic acid cycle. It is of interest in this connexion that many types of leucocyte have been shown to possess the ability to glycolyse in the presence of oxygen (Vannotti, 1961).

The less traumatic experiments of Hairston and Muller (1961) indicate that carbohydrate katabolism of the homotransplanted heart is in the normal range within short survival periods.

A further conclusion of Chiba *et al.* (1962) is that homograft rejection results in increased cell permeability with loss on enzymes and co-enzymes, although the respiratory chain appears to be intact. Our enzymological and analytical data partially support the concept of a loss of protein, including enzymes, from the tubular cells, but it is very unlikely that this alone would account for the enzyme reductions observed in homotransplants.

It is hoped that further investigations using serial biopsies from individual transplants will provide more precise information than that obtained by the experiments described here.

Summary

The respiratory enzymes succinic dehydrogenase and malic dehydrogenase, and the glycosidic enzymes β-glucuronidase, β-galactosidase, and β-glucosaminidase have been measured quantitatively in whole homogenates of normal, autotransplanted, and homotransplanted dog kidneys. Interpretation of the results is complicated by reason of variables such as the period of ischaemia, oedema, and the plasma-cell infiltration. Chemical analysis and enzyme histochemistry have assisted us in reaching the following conclusions:

(1) Autotransplants possessed slightly lower enzyme levels than normal kidneys. This is thought to be due to the period of ischaemia involved in the transplantation procedure.

(2) Homotransplants, removed within six days of transplantation, possessed lower enzyme levels than autotransplants—particularly in the case of dehydrogenases. These lower enzyme levels appeared to be independent of the period and degree of function. A change in the intracellular distribution of acid phosphatase was demonstrated histochemically in oliguric homotransplants; the reaction products being clumped in large masses rather than in small discrete granules.

(3) The further changes in enzyme levels observed in the long-surviving homotransplants (more than six days) can be explained by the effects of the massive interstitial cellular infiltration.

(4) Although the function of homotransplanted kidneys is abruptly terminated by the onset of an irreversible anuria, this preliminary biochemical investigation offers no immediate explanation of the cause of anuria.

The expenses involved in these experiments were defrayed by a grant to one of us (W. J. D.) from the Medical Research Council and the Wellcome Trust. We are indebted to Mr. M. V. Hounsome, Mr. R. Sparks, Miss Veronica Hanly, and Miss Valerie Brown for technical assistance.

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NATURAL HISTORY OF THYROID CARCINOMA

A STUDY OF 152 TREATED PATIENTS

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In order to try to piece together the natural history of thyroid carcinoma we have studied 152 patients with proved carcinoma of the thyroid who attended Hammersmith Hospital and King's College Hospital during the decade 1950–9. Since 62 of the patients were referred for radiotherapy, having had a biopsy or some kind of surgical excision elsewhere, this represents a highly selected group, and the incidence of carcinoma of the thyroid probably does not reflect the incidence of the disease in the general population. About one-third of these patients were included in a clinicopathological study reported by Alhadeff *et al.* (1956).

Carcinoma of the thyroid is not common, but it is of considerable interest and presents an unusual pattern of behaviour in that the younger the patient affected the better is the chance of survival. Although rare, it is important to diagnose the disease correctly as a variety of methods of treatment is now available.

Classification

For all practical purposes thyroid carcinoma can be considered as two separate diseases, so great are the differences between the anaplastic (undifferentiated) carcinoma on the one hand and the differentiated variety with its papillary and follicular patterns on the other. This fundamental observation was originally stressed by Dunhill (1931), and again more recently by Crile *et al.* (1948), Crile and Hazard (1953), and Crile (1957). It is convenient to subdivide the differentiated thyroid carcinomas into papillary and follicular after the manner of Warren and Meissner (1953), although this is to some extent an arbitrary division because it is usual to find papillary elements in most follicular carcinomas and follicular elements in the papillary ones. However, it is still possible to apply the term papillary or follicular according to which pattern has predominated in the number of fields examined under the microscope (Alhadeff *et al.*, 1956). Approximately two-fifths of our patients presented with the anaplastic type of carcinoma and the rest with differentiated lesions (Table I); this is a larger proportion of anaplastic lesions than in most published series (Table II), and is probably due to the fact that anaplastic tumours are especially suitable for radiotherapy and such patients were accordingly referred to the Medical Research Council's Radiotherapeutic Unit at Hammersmith Hospital. The size of these groups of patients allows comparison to be made between the behaviour of the different types of carcinoma.

It is not intended here to extend the arguments on histological interpretation of thyroid carcinoma, except to say that all attempts at classifying a disease must at times be somewhat arbitrary. In the case of thyroid carcinoma these difficulties have been exaggerated in the past because too much reliance has been placed on the histological appearances without reference to the general clinical picture, spread of the disease, and subsequent history of the patient. In addition due regard must be given to the response to treatment, the laboratory findings, and other investigations. A better appreciation of the natural history of the disease resolves many of the difficulties of classifying thyroid carcinoma.

TABLE I.—*Histological Type of Thyroid Carcinoma*

Type	Female	Male	Total
Follicular	33	10	43 } 86 (57%)
Papillary	29	14	43 }
Anaplastic ..	47	19	66 (43%)
Total ..	109	43	

Female: male ratio = 2·5: 1.

TABLE II.—*Published Incidence of Histological Types of Carcinoma*

Histological Type	Kilpatrick *et al.* (1957)	Franz and Yannopoulos (1961)	Lindsay (1960)	Woolner *et al.* (1960)
Follicular ..	45%	24%	30%	14%
Papillary ..	23%	58%	61%	60%
Anaplastic ..	30%	11%	9%	13%
Miscellaneous ..	2% (epidermoid carcinoma)	7% (solid, well differentiated)		10% (solid)

Thirteen unusual malignant lesions of the thyroid were seen during this 10-year period. Five of these were squamous carcinomas or tumours showing squamous metaplasia, seven were considered to be sarcomas, and one was a metastasis from carcinoma of the bronchus. It is possible that some of the so-called sarcomas were highly anaplastic carcinomas. There was also one example of "struma reticulosa" which conformed to the histological appearance and subsequent behaviour of some of the cases first described under this name by Brewer and Orr (1953). This patient, a woman aged 49, presented with a six-month history of swelling in the neck. Excision of the entire right lobe of the thyroid was performed, and it was reported as an anaplastic

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