subcultured every 6 to 10 weeks. Ten months after explantation, the technique of subcultivation was changed, since more rapid outgrowth of connective tissue was observed when explants were sandwiched between the wall of the roller tube and the slice of sponge. This has been our technique for growing strain D up to the present time.

The cells grow readily, as a latticework of spindle-shaped cells and collagenous fibers, forming a loose connective tissue throughout the sponge interstices. They also produce a dense layer of spindle-shaped cells on the outer surfaces of the sponge. Occasional bizarre, large cells have been seen from time to time, but the overwhelmingly predominant pattern has been one of a differentiated connective tissue in which collagenous fibers are readily produced. Four new subcultures may be made every 4 to 6 weeks from each culture tube. In practice, however, cultures are maintained for several months without subculture if new cultures are not needed for experimentation.

No known carcinogens have been added to the cultures, although on repeated replenishing of the medium, drops of nutrient adherent to the lips of the tube were charred on flaming, and occasionally fragments of this material were seen to drop into the tube as the rubber stopper was inserted. When the cell strain was 23 months old, one stock tube (D-189), a 15th subculture generation, which had been planted 4 months before, showed a remarkable pattern. Many large, bizarre cells were found extending from the edges of the sponge onto the adjacent glass wall. During the following 2 weeks the growth of pleomorphic cells became more extensive. The sponge was then removed, part of it was fixed in Zenker-formalin for histologic examination, and the remainder was subcultured.

Fig. 1. Microscopic section of a cellulose sponge tissue culture of strain D, in which the pattern of growth is that of a normal connective tissue. The cells are of uniform size and shape and do not appear malignant. Hematoxylin and eosin stain (× 125).

Fig. 2. Microscopic section of a culture of the spontaneously transformed connective tissue, strain D-189 (derived from strain D) in which the cytologic features of a malignant tumor are seen. Many cells are large and hyperchromatic. The nuclei in these are very large, and some are multilobate. Enormous nucleoli are seen in some cells. Hematoxylin and eosin stain (× 125).

The histologic pattern of the normal-appearing strain-D cell (Fig. 1) had been replaced in many areas on the outer surface of the sponge by a densely cellular tissue that appeared neoplastic. There were many large, hyperchromatic cells, some with single, large nuclei containing enormous nucleoli, others with four to six nuclei (Fig. 2). Dividing cells were numerous, and many of the mitotic figures were multipolar. The pattern seen in sections of this culture had the criteria on which the diagnosis of highly malignant anaplastic tumor is made. The altered tissue (D-189) in its fifth subculture generation since its recognition grows very rapidly.

The transformation of normal cells into malignant ones in tissue culture, with or without the addition of known carcinogens, has been observed in several laboratories in cultures of mouse and rat tissues (*2–4*). In each instance the malignant nature of the transformed cells was demonstrated by the production of sarcomas when the cultures were inoculated into animals of the same strain as the one from which the original cells had been obtained. It is not unexpected that human cells are capable of the same kind of transformation in tissue culture. In the instance reported here, the final proof of a malignant transformation, transplantation and growth in a genetically proper human host, is of course impossible. However, the biologic behavior of this new strain may be examined in animal transplant for a partial appraisal of its malignant properties.

Goldblatt and Cameron (*4*) have implicated anaerobiosis as a common factor in the production of malignancy *in vitro*. Since strain-D cells have been regularly covered by a blanket of cellulose sponge and plasma clot 0.5 to 1.0 mm thick on each subculturing for 13 months, anaerobiosis could have contributed to the formation of a morphologically malignant variant. Cellulose sponge itself may also be implicated as a causal factor, for Oppenheimer (*5*) has found that a variety of polymers including several cellophanes can induce sarcomas is the subcutaneous connective tissue of the rat.

Gey (*6*) has pioneered in the development of cell strains from malignant human tumors as well as of isogenic strains from normal areas of the same surgical specimen. Cell strains of such parallel origin may lend themselves to the discovery of distinctive metabolic properties of cancer cells.

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10 October 1955

## Human Arterial Hypertension: a State of Mild Chronic Hyperaldosteronism?

Many efforts in the search for a direct relationship between the adrenocorticoid hormones and arterial hypertension in human beings have met so far with no success. Chromatographic separation of urinary steroid extracts using an aluminum oxide column did not show any significant differences between normal and hypertensive subjects (*1*). However, the identification of aldosterone, a new adrenal mineralocorticoid hormone, by Simpson and Tait (*2*) has made it possible to restudy the problem with a more specific method (*3*).

Details of the assay procedure have been described previously (*4*). Only the main procedures will be outlined. Twenty-four hour urine aliquots adjusted to *p*H 1 were immediately extracted with four successive 1/5 portions of chloroform. The remaining urine was

reextracted similarly after readjustment to *p*H 4.5 and incubation for 24 hours at 37°C with animal β-glucuronidase (300 units/ml of urine). After two washings with 0.1*N* NaOH and one with distilled water, the combined chloroform extract was evaporated to dryness, then dissolved in 80-percent methanol and chromatographed on Whatman No. 2 paper in Zaffaroni's propylene glycol/toluene system for 96 hours at room temperature. The aldosterone-cortisone zone obtained was rechromatographed in the Bush C system (*5*) of toluene 90-ethylacetate at 10/50 percent aqueous methanol. The highly purified aldosterone fraction obtained after this second chromatography was eluted, evaporated to dryness, and kept in the deep-freeze until bioassay.

For each determination, five groups of eight to ten bilaterally adrenalectomized albino rats were used. One group served as control and received only the solvent (0.1 ml of aldehyde free ethanol). Two groups were injected with standard solutions of aldosterone (0.2 and 0.4 μg, respectively), and the last two groups received dilutions of the urinary aldosterone extract—one equivalent to the amount of urine excreted in 40 minutes, and the other to the output in 20 minutes.

The concentrations of sodium and potassium in urine after 4 hours' collection in the bladder were analyzed by the flame photometer. Aldosterone activity was measured both by the decrease in sodium excretion and reduction of urinary Na/K ratio relative to the excretions of the normal group.

In addition to five normal adult males, who served as controls, six patients with malignant hypertension and seven with severe essential hypertension (one of which could also be regarded as of renal origin) were studied. The criteria used in the diagnosis of malignant hypertension were papilledema, diastolic pressure above 130, diminished renal function, and poor general condition. Severe, but not malignant, cases of essential hypertension were those with persistent marked elevations of systolic and diastolic pressures, exudates and hemorrhages in the fundi, and signs of left ventricular strain. Diet was not controlled: the normal subjects were on their "ordinary" diet and the hypertensive patients received the regular ward diet. No restriction of sodium intake was made. No patients presented any endema.

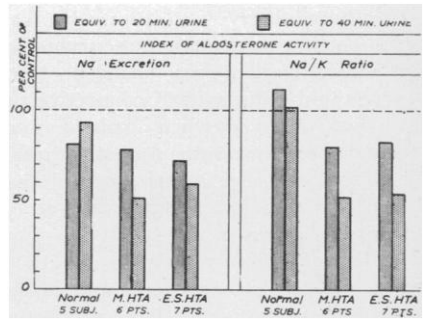


Fig. 1. Mean results of the three groups studied: five normal subjects, six patients with malignant hypertension, and seven with severe essential hypertension.

Table 1 shows the results of bioassay. The mean excretions of aldosterone obtained with doses equivalent to 20 and to 40 minutes of urinary output are shown in Fig. 1. With an extract equivalent to only 20 minutes of urinary output, the difference between the normal subjects and the two groups of hypertensive patients was not significant ($p > 0.1$). However, a difference between the two groups became evident and very significant in tests using extracts corresponding to 40 minutes of urinary output. The two indices of aldosterone activity, decrease in total Na excretion and fall in Na/K ratio, were quite concordant but unequally sensitive. Statistical analysis of the mean difference between normal subjects and hypertensive patients showed a $t$ value of 4.85 ($p < 0.001$) for the urinary Na/K ratio and a $t$ value of 3.27 ($p < 0.01$) for the Na index. The aldosterone equivalent (in micrograms per day) for each group of patients was calculated (*6*) with fiducial limits at $p = 0.05$ and is shown in Table 2.

These results suggest that hypertensive patients secrete relatively large amounts of aldosterone. Possibly this finding explains the abnormalities of sodium metabolism in these patients and the occurrence of hypertension in cases of adrenal hypercorticism. They also suggest that the human arterial hypertension could be caused by a state of mild and chronic hyperaldosteronism (*7*).

Table 1. Aldosterone in human urinary extracts. Bioassay results with adrenalectomized rats (percentage of control).

| Subjects | Aldosterone standards | | | | Equivalent | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.2 μg | | 0.4 μg | | 20 min | | 40 min | |
| | Na | Na/K | Na | Na/K | Na | Na/K | Na | Na/K |
| | | | | *Normal* | | | | |
| A.D. | 31.4 | 34.7 | 35.3 | 39.4 | 93.1 | 176.3 | 80.4 | 85.6 |
| E.K. | 31.4 | 34.7 | 35.3 | 39.4 | 76.5 | 114.8 | 85.3 | 105.5 |
| P.S. | 43.1 | 40.7 | 50.3 | 55.6 | 85.6 | 119.3 | 103 | 127.5 |
| J.G. | 63.5 | 56.3 | 30.8 | 31.6 | 90.6 | 109.6 | 66.3 | 84.4 |
| | (Aldost. 0.1) | | | | | | | |
| M.G. | 124.3 | 102.8 | 30.7 | 19.4 | 60.2 | 40.6 | 130.7 | 106 |
| | | | | *Malignant hypertension* | | | | |
| | (Aldost. 0.1) | | | | | | | |
| P.E.C. | 56.2 | 56.1 | 26.1 | 27.8 | 138 | 142.3 | 22.2 | 27.6 |
| G.R. | 63.5 | 56.3 | 30.8 | 31.6 | 47.6 | 62.1 | 67.2 | 77.1 |
| G.C. | 63.5 | 56.3 | 30.8 | 31.6 | 69.1 | 105.3 | 25.2 | 43.4 |
| M.J. | 36.8 | 25.1 | 38.1 | 30.5 | 57.8 | 23.2 | 72.3 | 41.8 |
| A.B. | 47.1 | 42.1 | 27.4 | 26 | 90.6 | 78.3 | 61.6 | 59.5 |
| G.M. | 40.78 | 36.34 | 13.40 | 28.3 | 66.48 | 68.5 | 54.74 | 64.2 |
| | | | | *Severe essential hypertension* | | | | |
| W.S. | 42.4 | 66.9 | 41.1 | 34.6 | 87.9 | 87.5 | 65.1 | 51.9 |
| J.J. | 91 | 82 | 40 | 35 | 125 | 142 | 85 | 69 |
| | (Aldost. 0.1) | | | | | | | |
| J.P. | 56.2 | 56.1 | 26.1 | 27.8 | 22.2 | 23.4 | 43.7 | 36.4 |
| | (Aldost. 0.1) | | | | | | | |
| S.B. | 56.2 | 56.1 | 26.1 | 27.8 | 39.8 | 45.1 | 20.9 | 17.8 |
| T.E.B. | 43.1 | 40.7 | 50.3 | 55.6 | 83.8 | 70 | 59.9 | 64 |
| J.A.D. | 88.46 | 79.3 | 47.1 | 39.7 | 116.34 | 107.7 | 76.9 | 74.54 |
| D.R. | 45 | 33 | 40 | 20 | 106 | 108 | 63 | 66 |

Table 2. Aldosterone equivalent for each group of patients (micrograms of aldosterone per day).

| Group | Na retention index | Decrease in Na/K ratio |
|---|---|---|
| Severe essential hypertension | 7.82 (F.L. 3.52–17.12) | 8.45 (F.L. 4.48–15.89) |
| Malignant hypertension | 7.2 (F.L. 3.74–13.75) | 5.47 (F.L. 2.19–13.94) |

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3. Work supported through grants of the Ministries of Health of Quebec and Ottawa (Federal-Provincial Plan), the Life Insurance Medical Research Fund, the National Research Council of Canada, and the Ciba Company, Montreal. We wish to express our gratitude for their collaboration and assistance to Fernande and Lucette Salvail, Pierrette Ledoux, Lise Langevin, Thérèse Ferguson, Aline Daoust, and Henry Schlegel; for calculations of the aldosterone equivalent to G. Russell, control laboratory, Ayerst, McKenna and Harrison Ltd., Montreal; for advice on statistical analysis and for many helpful suggestions to Vincent P. Dole, Rockefeller Institute, New York.


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21 September 1955

## Enhancement of Oxidative Phosphorylation of Glucose by Insulin

Polis *et al.* reported that under suitable conditions insulin enhanced oxidative phosphorylation of glucose by a preparation of liver mitochondria (*1*). This has been confirmed by us in experiments with rabbit tissue homogenates and extracts.

Rabbits were killed by stunning followed by decapitation, and the desired tissue (whole kidney or liver) was quickly removed and chilled on cracked ice for 2 to 3 minutes. The chilled tissue was homogenized in an all-glass (Pyrex) homogenizer (*2*) with 2 volumes of 0.067*M* phosphate buffer (Sorensen's) at *p*H 7.7 for 5 to 7 minutes at 0°C. The whole homogenate thus obtained was used in the majority of the experiments.

Table 1. Enhancement of oxidative phosphorylation of glucose by insulin in rabbit kidney homogenates and extracts.

| Expt. No. | Inorganic P esterified (mg) | |
|---|---|---|
| | Without insulin | With insulin (4 units/2 ml) |
| *Whole homogenates* | | |
| 1 | 0.98 | 1.10 |
| 2 | 0.53 | 0.65 |
| 3 | 0.80 | 0.90 |
| 4 | 0.90 | 0.98 |
| 5 | 0.96 | 1.10 |
| *Dialyzed extracts* | | |
| 6 | 0.82 | 0.91 |
| 7 | 0.51 | 0.59 |
| 8 | 0.42 | 0.56 |
| 9 | 0.50 | 0.55 |

In a few experiments, a dialyzed kidney extract was used. This was prepared as follows. Whole kidneys were homogenized as described in a previous sentence with 2 volumes of 0.1*M* phosphate buffer (Sorensen's) at *p*H 7.7. The homogenate was centrifuged at 2500 rev/min for 2 minutes and the turbid supernatant was dialyzed for 3 hours in a cellophane sack against 0.05*M* phosphate buffer at *p*H 7.7 and 1° to 2°C.

In experiments with whole homogenates, 1-ml portions of the homogenate were added to the main compartments of Warburg vessels that had previously been filled with 0.1 ml of 0.5*M* NaF, 0.2 ml of 0.5*M* sodium succinate and 0.5 ml of distilled water and standing on cracked ice. The side bulb contained 0.2 ml of 5-percent glucose. When the effect of insulin was tested, 0.1 ml of a 40-unit/ml plain insulin (Lilly) was added to the main compartment in the place of 0.1 ml of distilled water. The insulin was always added as the last addition. In experiments with dialyzed kidney extracts, 1-ml portions of which also were used in the experiments, the reaction mixtures contained, in addition to the afore-mentioned compounds, 0.1 ml of 0.5*M* $MgCl_2$ and 0.2 ml of 0.01*M* adenosine-5-phosphate.

Immediately after the additions had been made, the flasks were attached to the manometers and gassed with oxygen for 3 minutes at room temperature. They were then placed in the thermostat (38°C), and after temperature equilibration (7 minutes) the glucose in the side arm was tipped in and the flasks were shaken in the thermostat for another 20 minutes. The manometers were then taken out of the thermostat and the flasks were quickly immersed in ice water for a few minutes. The flasks were then detached, the protein was precipitated with trichloroacetic acid, and the material was filtered. The filtrates thus obtained were analyzed for their inorganic P content by the method of Fiske and SubbaRow (*3*) spectrophotometrically. The initial P contents of the reaction mixtures were determined in another set of flasks arranged and treated exactly in the same way as the experimental ones up to the stage of addition of glucose from the side arm.

Table 1 gives the results of the effects of insulin on oxidative phosphorylation of glucose by kidney homogenates and extracts. There was a small but definite increment in the amount of inorganic P esterified as a result of insulin treatment. Oxidative phosphorylation by the liver homogenates was also enhanced by insulin, and it was interesting to note that the enhancement was more pronounced with homogenates prepared from livers of alloxan-diabetic rabbits than with those prepared from the livers of normal rabbits (Table 2).

Table 2. Effect of insulin on oxidative phosphorylation of glucose by rabbit (normal and alloxan-diabetic, *4*) liver homogenates.

| Expt. No. | Inorganic P esterified (mg) | |
|---|---|---|
| | Without insulin | With insulin (4 units/2 ml) |
| *Normal rabbits* | | |
| 1 | 0.94 | 1.25 |
| 2 | 0.76 | 0.98 |
| 3 | 1.00 | 1.18 |
| 4 | 0.97 | 1.26 |
| 5 | 0.58 | 0.78 |
| 6 | 0.69 | 0.81 |
| *Alloxan-diabetic rabbits* | | |
| 7 | 0.35 | 0.50 |
| 8 | 0.64 | 1.04 |
| 9 | 0.73 | 1.25 |
| 10 | 0.64 | 1.14 |
| 11 | 0.63 | 1.05 |

In none of the experiments reported here did insulin have any effect on the oxygen consumption by the homogenates and extracts.


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4. Rabbits were made diabetic by injecting 200 mg/kg of alloxan intravenously. Many rabbits could not stand the initial complications of alloxan injection and died within 3 to 7 days after the injection. These rabbits stopped taking food and became extremely emaciated. Such rabbits were not used in the experiments. Some of the rabbits, however, while showing a strong diabetes, as evidenced by high glycosuria and hyperglycemia, survived the initial complications due to alloxan injection. They consumed food in amounts comparable to those consumed by the normal animals and were not very much emaciated. Such rabbits only were used in the experiments. We did not resort to insulin treatment to help the rabbits overcome the initial complications and death due to alloxan injection.


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12 September 1955

## Finding of Silver Positive Reticulum in Early Human Tubercles

While the importance of silver positive reticulum in the structure of the tubercle is accepted, the site and time of its earliest appearance and what ultimately happens to the reticulum are not entirely clear. The literature on the subject, (*1–4*) points to its occurrence, in man, only in formed tubercles. Experimental