

While it proved possible to demonstrate the  $\alpha$  and  $\beta$  cells in the pancreatic islets of the guinea pig, the rabbit and the ox by the differential staining technique of Gomori<sup>1</sup>, these cells could not be adequately differentiated in the pancreatic tissues of several normal sheep. In consequence, specific necrosis of the  $\beta$  cells could not be satisfactorily demonstrated in these diabetic sheep.

The data, however, prove unequivocally that the disordered intermediary metabolism typical of alloxan diabetes can be induced in a ruminant.

The details of this study will be published elsewhere.

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<sup>1</sup>Marston, H. R., "Ann. Rev. Biochem.", 564 (1939).

<sup>2</sup>Strand, B., Anderson, W., Alleroff, W., *Biochem. J.*, 28, 1, 642 (1934).

<sup>3</sup>Cutler, J., *J. Biol. Chem.*, 106, 653 (1934).

<sup>4</sup>Shaw Dunn, J., Sheehan, H., and McLetchie, N., *Lancet*, 1, 484 (1943).

<sup>5</sup>Bailey, C., and Bailey, O., *J. Amer. Med. Assoc.*, 122, 1165 (1943).

<sup>6</sup>Goldner, M., and Gomori, G., *Endocrin.*, 33, 297 (1943).

<sup>7</sup>Gomori, G., *Amer. J. Path.*, 17, 395 (1941).

is due to copper deficiency, some other factor must have affected copper metabolism. It is unlikely, however, since its content is within the usual range for pastures on which cases of swayback do not occur, that it is molybdenum which may be causing a conditioned copper deficiency in the lamb.

We feel that the above results should be made public at this stage in our investigations, lest the unsubstantiated suggestion quoted above should lead to the assumption that the primary cause of swayback in Britain is an excess of molybdenum in the herbage.

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<sup>1</sup>Dick, A. T., and Bull, L. B., *Aust. Vet. J.*, 21, 70 (1945).

<sup>2</sup>Bennetts, H. W., and Beck, A. B., *Counc. Sci. Ind. Res. (Austral.) Bull.* 147 (1942).

### Mechanism of the Proteolytic Activity of Malignant Tissue Cells

THE proteolytic activity displayed by malignant tumour cells *in vitro* has often been the main hindrance to cultivating permanent strains of these cells. The liquefaction of the culture medium by the malignant cells, which has attracted much attention, suggested to me years ago<sup>1</sup> that the proteolytic activity of the tumour cells was fully, or in part, responsible for their malignant properties. By the liquefaction the healthy tissue cells in the neighbourhood are deprived of their solid supports, and consequently the way is prepared for further infiltration deep into the normal tissues.

It therefore was important to know more about the mechanism of the sudden liquefaction which takes place in the plasma medium. Several years ago we found<sup>2</sup> that the digestion of the plasma clot occurs in the homologous medium only. So long as the cells digest the medium, it is impossible to measure the rate of growth. In order to compare the growth-rate of Rous chicken sarcoma cells with that of the normal chicken cells, both cell types had to be cultivated under identical conditions, namely, in a heterologous medium—in this case rabbit plasma.

The chicken sarcoma cells were found to grow without liquefaction throughout many passages, in a culture medium composed of rabbit plasma and chick embryo juice. When transferred to a chicken-plasma medium, liquefaction again takes place.

The liquefaction of the medium may be initiated in different ways: (1) by liberation of proteolytic enzymes from the living cells or after autolysis has taken place; (2) in contact with the demoenzymes on the surface of the cells; (3) by the activity of the enzymes contained in the blood plasma.

The following experimental observations have been made. When fresh chicken serum was added to the rabbit-plasma-medium, digestion of the plasma clot takes place to the same extent as in chicken plasma. If the chicken serum is first heated to 56° for 3-4 hours liquefaction does not take place. This indicates that the proteolytic enzymes in the surrounding medium must play an important part in the process. This was furthermore demonstrated in the following way. When the Rous sarcoma cells were cultivated in rabbit plasma to which rabbit serum, previously shaken with sand for half an hour, was added, digestion took place as readily as in the homologous chicken plasma.

The explanation of this experiment is probably that the proteolytic enzymes present in the rabbit-serum as inactive proenzymes have been activated by the shaking. In other words, the tumour cells and probably more or less all normal tissue cells seem to be able by contact to activate the proteolytic proenzymes in the homologous blood-plasma. Our experiments show that the activation is rather specific.

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<sup>1</sup>Fischer, A., *Arch. Entwicklungsmech.*, 104, 210 (1925).

<sup>2</sup>Fischer, A., and Laser, H., *Z. Krebsforsch.*, 26, 80 (1927).

### Aphosphorosis in Cattle in Offaly

THERE is evidence that in certain parts of Ireland a degree of mineral deficiency exists in old pastures, and, from time to time, isolated cases of disorders which respond to supplemental mineral feeding occur. A more pronounced condition of mineral deficiency is now reported from County Offaly. Mr. J. O'Donovan, county instructor in agriculture, and I are investigating the occurrence; and our results to date show that, over considerable stretches, particularly of the semi-moory type in that county, the old pastures supply insufficient phosphorus to meet the needs of the grazing animal.

Cows confined to such pastures develop a disorder known locally as 'hog crook', 'bog lame', 'rheumatism' or 'leg fever', the symptoms of which are pica, hidebound condition, staring coat, dulling of the skin colouring, reduction in milk yield, constipation, reduction in appetite and in body condition, abnormal posture when standing, difficulty in walking, lameness of one or more legs, a groan when moving and a stiffness and crackling in the joints. Temporary sterility usually sets in. Where the disorder persists, deformities manifest themselves in abnormal growth of hooves, swelling of the joints, stiffness of the backbone and neck with characteristic arching of the back and inability to raise the head to normal level. In chronic cases the extreme emaciation gives to affected stock the appearance of piners. In young cattle growth and development are retarded.

Experiments on the feeding of mineral supplements show that in all cases so far investigated—some forty cows have been on test—the deficiency is one of phosphorus, the supplementary feeding of which is as effective as that of a complete mineral mixture. The feeding of sterilized bone flour at the rate of 2 oz. a day, or meat and bone meal (containing 34 per cent of calcium phosphate) at the rate of  $\frac{1}{2}$  lb. a day causes alleviation of the disordered condition within a few weeks, and completely restores affected animals to normal appearance and health and reproductive activity in six to twelve weeks. It is recommended that the sterilized bone flour and meat and bone meal be mixed with salt and with such palatable foods as sugar beet, bran, or oats in order to induce animals to consume them readily.

The incidence of the disorder is worst during the grazing season; but the symptoms are less pronounced when the pasture herbage is supplemented by meals, especially by bran, the efficacy of which is, no doubt, connected with the comparative richness of these foods in phosphorus.

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### Molybdenum and Copper Metabolism of Farm Animals

In a recent communication from Australia by Dick and Bull<sup>1</sup> the view was expressed that "an increase in the molybdenum content of the pasture may possibly explain the seeming anomaly of a copper deficiency in sheep, for example, grazing on pastures which show by ordinary chemical analysis a copper concentration within normal limits". Since the references are to work in Britain in connexion with a disease of lambs known as 'swayback', the following may be of interest.

In 1943 there was a large increase in the incidence of swayback in lamb flocks throughout Scotland, some flock owners observing this condition for the first time in their lambs and others noting a tenfold increase in incidence. Pasture and soil samples were obtained from three different areas of Scotland—Ross-shire, Perthshire and Midlothian—and were analysed spectrophotically. Since a copper therapy had been in vogue for several years in cases of 'teart' in cows, known to be associated with a high content of molybdenum in the pasture, determinations of molybdenum in addition to other trace elements were made.

The copper content of the ten samples examined varied from 4.4 to 9.2 p.p.m., while the molybdenum contents varied from 0.34 to 1.20 p.p.m., the respective values being:

Cu (p.p.m.) 8.8 8.9 8.6 7.0 6.6 6.2 4.4 4.6 5.7 9.2  
Mo (p.p.m.) 0.95 1.07 0.94 0.44 0.45 0.34 0.60 0.70 1.00 1.20

Thus, while the copper contents cover values which are marginal (less than 5 p.p.m.) to normal as defined by Bennetts and Beck<sup>2</sup>, the molybdenum contents are in all instances normal. Where the copper content exceeds 5 p.p.m., it would appear that, if swayback

### Relation of Intracellular Potassium to the Refractory Period of the Frog's Heart

IT has been known for some time that vagus stimulation and acetylcholine decrease the refractory period of the frog's heart so that the muscle may be tetanized<sup>1</sup>. A similar tendency to tetanization has recently been found in normal hearts<sup>2</sup>.

During experiments in this laboratory on removal of potassium from the cells of frogs' hearts, it has been found that decrease of intracellular potassium first produces a holding of contraction with slowing of relaxation, followed by decreased contraction and quickening of relaxation<sup>3</sup>.

Measurements of refractory period on hearts treated with atropine-ergotoxine showed that it increased in the first phase and decreased to 30 per cent of normal in the second phase. In the second phase, an extra stimulus just outside the refractory period causes a much increased contraction (see record). For this increased contraction two factors are essential: (a) reduced refractory period, (b) a block (or blocks) the passage of which is facilitated by the first stimulus, so that the excitation due to the second stimulus gets through—whether the block is one of conduction or one between excitation and contraction mechanisms is not yet clear. The facilitation is maximal at the end of the refractory period and falls to a minimum in three seconds,