has been reported by Anderson (1951): five out of eight siblings developed leukaemia when 5 to 8 years old.

Vertical transmission of potentially pathogenic agents should not be confused with inheritance. The differentiation is not always easy. When observing a disease appearing in several descendants of successive generations within the same family of hosts, a casual observer may consider such cases to be caused by some obscure inherited genetic factors. Yet inheritance is conditioned by genetic factors, transmitted in an orderly and foreseeable manner, and is not acquired. Vertically transmitted diseases, on the other hand, are caused by viral agents, and represent a form of infection. The potentially pathogenic agents had been most probably at one time acquired, even if this might have happened in individual families many thousands of years ago; transmitted, since that time, from one generation of their hosts to another, invisible and completely adapted, such agents represent examples of "perfect parasites," causing in most instances no harm to their hosts. Only now and then, prompted by obscure reasons, they may change into formidable pathogens, causing symptoms of fatal disease and destroying their hosts. Such "accidents," however, would be usually separated by several generations of apparently healthy carrier-hosts.

It is quite possible that not only in animal species but also in humans malignant tumours, including the various forms of leukaemias, are caused by oncogenic agents seeded in millions of apparently healthy hosts and transmitted from one generation to another (Gross, 1954).

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EFFECT OF INSULIN AND GLUCAGON
ON TUMOUR GROWTH

BY

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During the past few years we have been working on certain problems related to both insulin and glucagon (Salter and Best, 1953; Salter *et al.*, 1957a, 1957b; Davidson *et al.*, 1957). There is considerable evidence in the earlier literature that insulin may cause some inhibition in the growth of malignant tissue (Piccaluga and Cioffari, 1925; von Witzleben, 1925; Silberstein *et al.*, 1927; Gomes da Costa, 1931).† The results of experiments carried out in this laboratory are in general agreement with these earlier reports. We find that, although the effects of giving insulin to rats bearing the Walker carcino-sarcoma vary considerably, a small but significant inhibition of the growth of this tumour is produced in most instances. The findings presented in this preliminary communication show, for the first time, that glucagon alone also produces a significant inhibition in the growth of this tumour, while the simultaneous treatment with both glucagon and insulin consistently causes an inhibition that is considerably greater than the sum of their individual effects.

Methods

Female Wistar rats weighing approximately 150 g. were used in all experiments. The animals were fed powdered Purina chow containing 5% corn oil.

Walker carcino-sarcoma tissue was collected from several donor rats, pooled and macerated with three parts (by weight) of 0.9% saline until the resultant brei would pass through a 23-gauge hypodermic needle. Each animal was then injected in both gastrocnemii with 0.2 ml. of the suspension. Treatments were started three days after the inoculations. At this stage the tumours were just palpable. In every experiment all rats had palpable tumours at the start. Protamine-zinc insulin (P.Z.I.) was injected once daily at 3 p.m. Crystalline glucagon suspended in 0.9% saline was administered subcutaneously at 9 a.m., 3 p.m., and 9 p.m. The rats were sacrificed after 10 to 15 days of treatment. The back legs were skinned, carefully disarticulated at the hip-joint, and removed. Tumour weights were determined by subtracting from the weight of the tumour-bearing leg the average weight of the normal hind limb in rats of the same size. The doses of insulin and glucagon and other relevant data are given in the Table.

Results

The results of the experiments are also presented in the Table. In Experiment 1 the administration of insulin inhibited the growth of the tumour by 10%, while in

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†Our former colleague, Dr. Spencer Monroe, now of the Sloan-Kettering Institute for Cancer Research, has recently discussed with us his observations on the dramatic inhibition by insulin of the growth of Rous sarcoma in chickens.

Experiment 5 no inhibition was discernible. In a large series of other trials not reported here, insulin consistently produced a small but significant slowing of the tumour growth. This inhibition occurred only when the food intake of the treated animals was restricted to the level of the controls.

The retardation of growth of the tumour induced by glucagon alone has varied from 20 to 40%. This inhibition can be attributed, in part, to the reduction in food intake and the adverse effect of glucagon on the growth of the rat as a whole (Salter *et al.*, 1957b). Although the rats treated with both insulin and glucagon lose less weight than animals given glucagon alone, inhibitions of tumour growth of 60–70% have been obtained invariably. The inhibition is not therefore proportional to the weight loss. In each experiment the carcinostatic action of the combined therapy has been much greater than the sum of the individual effects of the two substances.

Discussion

The data show that both insulin and glucagon produce some inhibition, which is, on the average, about 10% for insulin and approximately 30% for glucagon. Together

that a prolonged remission may have taken place, but there is no proof as yet that this can actually be made to happen.

Hypoglycaemia still presents a serious problem, but the experience we have gained enables us to control the level of blood sugar better than was possible initially. A long-acting glucagon would be of great value in these studies.

If we consider for the moment only the control animals and those which have received insulin and glucagon together there have been in all 67 controls and 77 treated with the two substances. The inhibition of tumour growth produced by glucagon and insulin together has been remarkably consistent, but a great deal of further work will have to be done on this tumour and on others to determine the maximum extent and duration of the inhibition and the frequency with which it takes place in various species of animals. Dr. E. S. Goranson, of the Ontario Cancer Institute, working with one of us (J. M. S.), has already repeated our experiment, and has found that an extensive inhibition (80%) is exerted by insulin and glucagon together on the growth of the Novikoff tumour in rats. There was a negligible difference in the weight of the control and test animals at the end of the experiments. Studies of various other tumours are under way.

FIG. 1.—The upper animal was treated with both glucagon and insulin. The tumours present in the back legs are barely discernible, while those in the control animal, immediately below, are obviously much larger.

FIG. 2.—The tumours of the rats from Fig. 1 are shown after surgical exposure (circled in white). The tumour on the right is from the glucagon-insulin treated rat. The inhibition evident here is slightly greater than usual, but we have seen even more pronounced inhibition in some instances.

they exert from 60 to 70% inhibition of growth of this particular tumour (Figs. 1 and 2).

We have thus far only established that the inhibition persists while insulin and glucagon are being administered. Cessation of treatment is followed by resumption of growth of the tumour. There has been a suggestion in a few cases

It is abundantly clear that these findings demand, and that they will probably stimulate, an even more vigorous exploration of the metabolic effects of insulin and glucagon given together. No attempt will be made here to review the controversial literature on this subject. If glucagon supplements the peripheral action of insulin on normal

Results of Experiments

Exp. No.	Treatment	No. of Animals		Days' Duration	Av. Daily Food Intake (g.)	Final Wt. (g.)	Wt. Change (g.)	Tumour Wt. (g. S.E.)	Inhibition (%)
		Initial	Final						
1	None	25	25	10	12.8	156	+14	13.4 ± 0.42	10
	P.Z.I. (1.6 u./day)	50	35		12.8*	154	-16	12.0 ± 0.33	
2	None	25	25	12	10.3*	151	+3	16.4 ± 1.00	20
	Glucagon (150 µg./day)	25	25		10.3	142	-10	13.1 ± 0.78	
3	None	25	20	15	9.5*	138	-6	18.2 ± 2.10	27
	Glucagon (300 µg./day)	20	20		6.8	111	-29	13.2 ± 1.00	
4	None	20	20	10	9.5	129	-13	6.1 ± 1.18	67
	Glucagon (300 µg. + P.Z.I. 3 u./day)	50	28†		10.5*	148	-5	13.5 ± 1.67	
5	None	20	20	10	5.6	116	-39	9.2 ± 0.88	32
	Glucagon (900 µg./day)	20	20		10.5	132	-18	5.4 ± 1.35	
6	None	27	27	10	10.1	159	-10	7.0 ± 1.12	40
	P.Z.I. (1.6 u./day)	30	16		11.6*	150	-3	7.0 ± 0.81	
7	None	25	22	10	9.4	121	-33	4.1 ± 0.93	70
	Glucagon (210 µg./day)	34	24†		10.3	130	-18	2.1 ± 0.48	

* Food intake restricted to this amount. † All deaths due to hypoglycaemia.

Figure represents weight of carcass exclusive of tumour.

tissue, the effects of the two together in "starving" the tumour may be supplementary. Equally plausible suggestions may be based on the possible katabolic effects of glucagon on the tumour itself. Both mechanisms could be involved.

The field of tumour growth is notorious for the frequency of pitfalls. We have seen no indication that the effect we are investigating is due to a contaminating infectious agent or to other artifacts. These may, of course, emerge later and necessitate a change in the interpretation of our findings. We have obtained glucagon from two sources—Indianapolis and Toronto. The various lots of insulin have been made in Toronto.

It is obvious that, if this phenomenon of the extensive inhibition of tumour growth by insulin and glucagon together is observed in a large number of other tumours in various species of animals, a clinical trial of the substances may become very tempting. Some aspects of the investigation would be easier to control in the hospital than in the experimental laboratory. Clinically the two substances *could* be given by continuous intravenous drip and the hypoglycaemia which has plagued us could be detected at an early stage. Frequent blood-sugar estimations have not been considered advisable in the small animals. A sufficient number to establish low blood sugar as the cause of death in the insulin-treated animals which succumbed have been made. A great deal more should be learned in experiments on animals before any clinical study is seriously considered. An inhibition which persists only during the administration of the substances would be of limited clinical interest. The obvious possibility of using other substances to supplement the inhibition produced by insulin and glucagon is being explored.

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Dr. A. J. DALZELL WARD, medical director of the Central Council for Health Education, said at a recent press conference on the dangers of the common housefly, "Eggs laid last autumn are now hatching out. It takes about 12 days for the egg to hatch and another 12 days to reach maturity. During its brief life the housefly can lay about 900 eggs. Within a couple of months or two generations, therefore, some 800,000 (certainly half a million) flies can come from one fly. In other words one fly can have 800,000 grandchildren." Dr. Ward went on to describe prophylactic measures against the housefly. Obviously the best way to stop this menace to health was to prevent them breeding, he said. The lids of dustbins should be tight and not battered. The bottoms of dustbins should be kept clean and dry, and if possible sprayed with insecticide, preferably one containing D.D.T. It was a good thing to line the bottoms of dustbins with newspaper and renew this whenever they were emptied. As much rubbish as possible should be burned and the remainder, if burning was not possible, kept wrapped and covered until disposed of.

The housefly could and did travel as much as 10 or 12 miles (16–20 km.) from its breeding-place.

OOPHORECTOMY IN BREAST CANCER AN ATTEMPT TO CORRELATE CLINICAL RESULTS WITH OESTROGEN PRODUCTION

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The treatment of metastatic breast cancer by surgical castration is the oldest rational method of palliation for this disease (Beatson, 1896). In the series reported by Thomson (1902) and Lett (1905) objective signs of growth regression in pre-menopausal women were observed in 25% of cases. On the other hand, and in sharp contrast, growth regression was limited to a single instance in a series of 25 post-menopausal women.

From 1910 onwards the usefulness of surgical castration was seriously questioned. For over thirty years it was generally discredited, and it was not reinstated until 1942 (Farrow and Adair, 1942) and Beatson's original and cautious claims were confirmed (Adair *et al.*, 1945; Sicard, 1947; Dargent, 1949). Objective remissions in post-menopausal cancer were again shown to be very uncommon.

The therapeutic purpose of surgical castration in pre-menopausal breast cancer is to arrest the progress of the disease by removing the source of ovarian oestrogen production. The palliation induced by this surgical manoeuvre is clearly and intimately related to the regressions which are frequently observed during the "physiological castration" of a natural menopause in breast cancers which, arising during the pre-menopausal period, have already metastasized by the time the patient enters the menopausal phase (Clemmesen, 1948; Hadfield and Holt, 1956).

Laboratory studies of the effectiveness of oophorectomy in abolishing oestrogen production have not been numerous. Bio-assays or biochemical estimations of urinary oestrogens have been reported by Smith and Emerson (1954), Dao (1953), Bulbrook and Greenwood (1957), and McBride (1957), and studies of vaginal cytology were published by Struthers (1956). These investigations revealed that, whilst surgical castration does not abolish the excretion of oestrogens, it does significantly reduce the excretion in pre-menopausal women.

None of the above authors attempted to correlate the immediate and long-term fluctuations in urinary oestrogens with the clinical consequences of the operation. In the present communication this correlation has been attempted.

Material and Methods

All clinical regressions recorded have been based exclusively on observable objective improvement in superficial lesions or in deep lesions visualized by radio-graphy, and all clinical assessments were made without prior knowledge of the biochemical estimations.

Oestrogen-excretion studies were carried out in hospital. Patients were divided into two groups. Those