BRITISH MEDICAL JOURNAL

LONDON SATURDAY JULY 5 1958

THE AETIOLOGY OF CANCER AND ALLIED DISEASES DEVELOPMENT OF A CONCEPT BASED ON RECENT EXPERIMENTS DEALING WITH A CELL-FREE TRANSMISSION OF MOUSE LEUKAEMIA

BY

LUDWIK GROSS, M.D., F.A.C.P.

From the Cancer Research Unit, Veterans Administration Hospital, Bronx, N.Y., U.S.A.

It has long been suspected that cancer and allied diseases may be caused by transmissible viruses. This concept appeared particularly plausible as a hypothetical explanation of the obscure aetiology of various forms of leukaemias, lymphosarcomas, and Hodgkin's disease. The course of either disease in humans with the accompanying fever; the frequent temporary improvements followed by relapses; the general systemic manifestations, including the gradual loss of weight and anaemia; the striking, though rare, reports of familial incidence in humans and in animal hosts, were consistent with the assumption of a chronic infectious nature of these diseases.

The main difficulty, however, in accepting the infectious concept of the aetiology of malignant tumours, including leukaemias, has been the well-established observation that in humans, and in animal hosts, tumours are not transmissible from one host to another, at least not in a manner similar to that observed in other infectious diseases. When speaking of a communicable disease we are conditioned to think automatically of a disease spreading from one host to another within the same generation; by contact (smallpox), through infected food or carriers (typhoid fever), through airborne droplets (common cold, influenza), etc. In each case both the infecting and the infected hosts belong to the same generation; a brief interval of time, most frequently not exceeding a few days, or weeks at the utmost, elapses between the individual cases forming successive links in the chain of a conventional transmission of the common communicable disease.

In the typical cases the human observer can actually follow the spread of a communicable disease, tracing it in either direction, back to the infecting carrier, or ahead to the infected host. Thus, a child suffering from chicken-pox, or measles, for instance, could readily cause a rapidly spreading epidemic, progressing from one child to another in the neighbourhood. Similarly, a mouse infected with the salmonella organism would spread typhoid rapidly to other mice within the same cage. Malignant tumours, including the various forms of leukaemias, are obviously not transmissible in such a manner. A leukaemic or sarcomatous chicken could be placed for the balance of its life in close quarters with a flock of healthy chickens; a leukaemic mouse, or a mouse bearing a large, perhaps even ulcerated mam-

mary carcinoma, could be placed in the same cage with a group of healthy mice; yet no transmission of the disease would occur in either case. A similar observation has been repeatedly made, over the period of decades, in human tumours and leukaemias. It appeared logical to conclude, therefore, that malignant tumours, including leukaemias, are not transmissible diseases.

Thus the viral theories of cancer, advanced now and then during the past decades, appeared to be doomed at the onset, and fell in disrepute when confronted with common observations in clinical medicine and experimental laboratories. Actually, however, upon reviewing clinical and experimental observations thus far accumulated, it does not seem necessary to conclude that cancer and leukaemias are not caused by transmissible agents. It can only be concluded that malignant tumours do not appear to be transmitted from one host to another in the conventional manner hitherto observed in the common communicable diseases.

"Vertical" Transmission of Oncogenic Agents

The transmission of oncogenic agents from one host to another may take place in a different manner, not usually observed in the common infectious diseases. Instead of spreading "horizontally" among hosts of the same generation, a potentially pathogenic agent may instead be transmitted from a carrier host to the host's offspring, thence to the offspring's offspring, thence, in turn, to the next generation, and so forth. Such a transmission would obviously require much more time than that observed in those communicable diseases which spread in the conventional manner within the same generation. The term "vertical transmission" or "vertical epidemic" was suggested (Gross, 1944) to designate the transmission of potentially pathogenic agents from one generation to another, usually through germinal cells, occasionally through mothers' milk, as in the case of mouse mammary carcinoma (Bittner, 1936). The agent, though potentially pathogenic, would remain latent for a long period of time, often perhaps throughout the entire life-span of a given carrier host. During such an interval of time the potentially pathogenic agent would remain frugal and moderate in its requirements, causing no harm to its carrier host. Thus the host would remain in perfect health, while carrying and transmitting the seeds of the disease.

In some of the hosts, however, particularly in those reaching middle age, the agent may become activated. Triggered by intrinsic or extrinsic stimuli, the hitherto latent and harmless agent would then change into a formidable pathogen, causing rapid multiplication of

*Special University Lecture in Pathology delivered at the London School of Hygiene and Tropical Medicine on May 16, 1958, entitled "Viral Aetiology of 'Spontaneous' Mouse Leukaemia: its Possible Implications for the Problem of Cancer." Aided, in part, by grants from the Damon Runyon Memorial Fund and the American Cancer Society.

cells harbouring it, and killing its carrier. In most instances, however, activation of the agents would not occur. The hosts would remain in good health, even though they would carry the agent and transmit it to their offspring.

The triggering factors responsible for the activation of the hitherto latent agents would be of varied nature, generally non-specific. Essentially, these triggers would be either intrinsic (metabolic or hormonal) or extrinsic (such as x rays and other forms of ionizing radiation energy, certain chemical poisons, physical factors such as prolonged irritation, etc.).

Viral Aetiology of Cancer and Leukaemia

On the basis of this working hypothesis, one could regard the development of a malignant tumour or that of leukaemia as the result of an activation, perhaps accidental, of oncogenic agents, hitherto latent, and carried by the host. Fortunately, in most instances no activation of the oncogenic agents would occur during the life-span of the host, even though such agents would be transmitted to the host's offspring. Thus only now and then malignant tumours or leukaemia would develop in some of the members of those families in which the agents would be transmitted from one generation to another. Such instances, however, representing only unhappy "accidents" in the otherwise harmonious relationship between the host and its usually well adjusted and latent oncogenic agents, would often be separated by two or three generations of healthy carrier individuals. In humans, therefore, two cases of leukaemia or other malignant tumours representing successive links in the generation-to-generation transmission of the same oncogenic agent might frequently be separated by a century or two. It is quite obvious that the tracing of the "vertical" host-to-host transmission of oncogenic agents in man would be extremely difficult.

The concept of vertical transmission of oncogenic agents may be new as applied to tumours. Actually, however, there exist in nature numerous examples of pathogenic agents transmitted from one generation to another. A virus disease of the silkworm, pebrine, is transmitted through the eggs from one generation to another (Pasteur, 1865). The mosaic disease of lettuce, tomato, or potatoes is caused by viruses transmitted from one generation to another through the seeds, or in the case of potatoes through the tubers; these viruses may remain latent for long periods of time (Kunkel, 1928). The infected plant may grow next to a healthy one, yet no transmission of the virus will occur among hosts of the same generation, unless an intermediary carrier, such as an insect vector, is interposed. In certain instances the vertically transmitted agents may be so adapted to their own hosts that they will not cause harm to their own carriers but may become pathogenic when transmitted to other breed or species. The Rocky Mountain spotted-fever rickettsiae, or the tularaemia organisms, for instance, are transmitted in the tick, through the eggs of these insects, from one generation to another. They cause no harm to their carrier hosts, but may become pathogenic and cause disease when transferred to other species, such as humans.

The temperate phage may also be transmitted from one generation to another in certain lysogenic bacterial clones (Lwoff, 1953). The phage may remain perfectly harmless to its hosts, as a prophage. In very rare instances it may spontaneously become pathogenic, destroying its own host by lysis. The activation may be prompted at will, however, by certain inducing agents, such as ultra-violet (Lwoff *et al.*, 1950) or x-ray irradiation (Latarjet, 1951).

It is obvious that the vertically transmitted pathogenic agents can readily escape detection, since they are submicroscopic, and since they only occasionally cause symptoms of disease.

Transmission of Tumours by Filtrates

It is difficult to determine the nature of an obscure disease with the rather limited means available in an experimental laboratory. One of the first steps consists usually in an attempt to transmit the disease from one host to another.

It should be stressed that there are no particular criteria to indicate, *a priori*, whether a given disease is caused by a transmissible agent. In many instances, when studying a new and obscure disease, the investigator may be confronted with symptoms appearing to develop "spontaneously." Yet the disease may be communicable, even though it may be quite difficult to trace the mode of transmission of the pathogenic agent. If experimental transmission succeeds in the laboratory by means of inoculation of filtrates, it can be assumed that the investigated disease is actually caused by a transmissible filterable agent, presumably of viral nature. It still remains to be determined, however, how the actual transmission of the agent occurs under natural conditions of life (Tables I and II).

TABLE I.—Transmission of Tumours by Filterable Viruses in Animals

1898 Rabbit myxomatosis	Sanarelli
1908 Chicken leukaemia (erythromyeloblastosis)	Ellermann and Bang
1911 Chicken sarcoma	Rous
1932 Rabbit fibroma	Shope
1932 Dog oral papilloma	DeMonbreun and Goodpasture
1933 Rabbit papilloma	Shope
1933 Chicken lymphomatosis	Furth and Breedis
1934 Dog lymphosarcoma	DeMonbreun and Goodpasture
1936 Mouse mammary carcinoma	Bittner
1936 Rabbit oral papilloma	Parsons and Kidd
1938 Frog kidney carcinoma	Lucke
1946 Chicken lymphoid tumours and osteopetrosis	Burmester <i>et al.</i>
1951 Mouse leukaemia	Gross
1951 Horse cutaneous papilloma	Cook and Olson
1953 Mouse parotid carcinoma	Gross
1955 Mouse sarcoma	Gross
1955 Deer fibroma	Shope
1957 Swiss mouse leukaemia	Friend
1958 Hamster sarcoma	Eddy <i>et al.</i>

TABLE II.—Transmission of Human Tumours from Man to Man by Filtered Extracts

1907 Warts	Ciuffo
1919 Warts	Wile and Kingery
1917 Condyloma acuminatum*	Waelisch
1923 Laryngeal papilloma	Ullmann

* Extract not filtered.

The discovery by Ellermann and Bang in 1908 that leukaemia in chickens could be transmitted by cell-free extracts provided the first experimental support of the concept of viral nature of leukaemia. Even though the cell-free transmission was accomplished only in the fowl, it strengthened the suspicion that in mammals also leukaemia may be caused by similar filterable agents.

"Spontaneous" Mouse Leukaemia

Leukaemia occurs as a spontaneous disease in various strains of mice. In some strains, such as C3H or C57 Brown, it develops only occasionally. In other strains it is more frequent, and in two outstanding inbred strains of mice—namely, C58 and Ak—leukaemia develops spontaneously in up to 90% of middle-aged animals. Over 25 years ago mice of the strain designated by the symbol C58, and bred by MacDowell and Ritcher (1935), were observed to die with large spleens; it was then realized that mice of this particular strain develop leukaemia spontaneously. On the other hand, Furth and his associates (1933) developed purposely a high leukaemic strain of mice by breeding selectively relatives of leukaemic mice and continuing such selective inbreeding through a number of successive generations; they designated this particular leukaemic strain by the symbol Ak. Leukaemia, usually of the lymphatic type, develops in middle-aged Ak mice of both sexes, though the onset begins slightly earlier in females. The incidence of spontaneous leukaemia in a sample of 500 Ak females was in our laboratory 94% at 8.6 average age (Gross, 1958b). It begins to develop approximately at 4 to 4½ months of

age; this incidence rises rapidly, reaching its peak at 7 to 9 months, then declines gradually.

The development of spontaneous leukaemia in over 85% of middle-aged mice in certain inbred lines of mice, such as C58 or Ak, raised immediately the question whether a virus transmitted from one generation to another in certain families of mice may not be actually responsible for the development of this disease. The existence of a leukaemic agent, though long suspected, was not established, however, until the experimental conditions were recognized under which mouse leukaemia could be transmitted by filtrates. All previous attempts to transmit mouse leukaemia by filtered extracts failed prior to 1951 (Gross, 1958b). Transmission of leukaemia in mice appeared possible only when live cells were transplanted from a leukaemic mouse to another host usually of the same or of a genetically related inbred line.

Transmission of Mouse Leukaemia by Filtrates Inoculated into Newborn Mice

A few years ago it was unexpectedly found in our laboratory (Gross, 1951a) that naturally occurring "spontaneous" mouse leukaemia could be transmitted by filtered extracts, provided that certain experimental conditions were met. Thus, newborn, less than 16 hours old, mice of a low leukaemic but susceptible inbred strain—namely, the C3H inbred line (with an incidence of spontaneous leukaemia less than 0.5%)—could be successfully inoculated with filtered extracts prepared from spontaneous Ak leukaemia.

Leukaemia developing in C3H mice following inoculation of the leukaemic filtrates was usually of the lymphatic type, and resembled essentially that which develops spontaneously in mice of the Ak strain. Of a total of 328 newborn C3H mice inoculated with filtrates, 28% developed leukaemia at an average age of 10 months. This compared with an incidence of less than 1% among 166 littermate controls (sister and brothers), either not treated or inoculated simultaneously with heated (65° to 68° C. for half an hour) leukaemic extracts, and surviving to an average of 16 months (Gross, 1951-8).

Surprisingly, among the inoculated C3H mice, some developed, instead of leukaemia, bilateral parotid gland carcinomas, and a few developed subcutaneous fibrosarcomas (Gross, 1953). These results were later confirmed and extended by Woolley and Small (1956), Furth *et al.* (1956), Rudali *et al.* (1956), Dulaney *et al.* (1957), Hays *et al.* (1957), and Kassel and Rottino (1958).

One of the initial difficulties in transmitting mouse leukaemia by filtrates was due to the difference in susceptibility among the strains used for inoculation of the extracts (Gross, 1955); furthermore, it soon became evident that the results of individual experiments may vary to a considerable degree, depending on the extract used. Of 70 leukaemic filtrates, each prepared from a different donor, and tested in our laboratory, 18 were found to be completely inactive on inoculation tests (Gross, 1957b).

Selecting a particularly potent extract, and passing the agent serially through several successive inoculations of newborn hosts, made it recently possible to develop a very potent filterable mouse leukaemia strain (Gross, 1957a). Filtrates prepared from this passage virus can now be inoculated into suckling C3H mice 1 to 14 days old, inducing leukaemia in up to 97% of the inoculated mice after a latency of only three to four months (Gross, 1958a).

It is quite interesting that leukaemic filtrates inoculated into C3H mice less than 16 hours old induce leukaemia, and in some instances parotid gland tumours, whereas the same filtrates inoculated into suckling C3H mice 1 to 14 days old induce leukaemia but not parotid tumours (Gross, 1958a). It appears, therefore, that in order to induce parotid tumours, newborn, less than 16-hours-old, C3H mice have to be inoculated with the leukaemic extracts.

Previous attempts to transmit Ak leukaemia by inoculation of filtrates into adult C3H mice were seldom successful. After the potency of the leukaemic agent has been increased

through serial cell-free passage, it is now possible, however, to induce leukaemia also by inoculating passage leukaemic filtrates into adult C3H mice; the incidence of induced leukaemia is approximately 37% after a latency of seven months (Gross, 1958a). Suckling, 1 to 14-days-old, C3H mice, however, are substantially more susceptible to the inoculation of the leukaemic filtrates than adult hosts.

It is apparent, therefore, that the induction of different types of tumours requires the use of a suitable recipient strain for inoculation of the leukaemic extracts. Moreover, the cell-free extracts must be inoculated into newborn mice less than 16 hours old, if the full array of the different types of tumours is to be induced.

The development of leukaemia in some of the mice inoculated with the filtrates, and that of parotid carcinomas or subcutaneous fibromyxosarcomas in others, raised immediately the fundamental question whether a single agent may be able to cause different types of tumours, or whether several distinct agents were present in the leukaemic extracts.

One could assume that one agent may be able to induce different types of tumours. It is also possible to speculate, however, that certain tumours, at least, may carry, either accidentally or for a good reason, a number of distinct oncogenic agents. Under proper experimental conditions some of these hitherto latent agents may assert themselves, inducing a wide spectrum of different tumours. Such a hypothesis would explain the puzzling results of experiments reported by Graffi *et al.* (1956) and Friend (1957).

Development of Leukaemia Following Inoculation of Newborn Mice with Cell-free Extracts from Transplanted Mouse Tumours

The use of newborn, less than 1 day old, mice for inoculation of oncogenic agents, so successful in studies on cell-free transmission of mouse leukaemia (Gross, 1951-3), soon became a useful tool, and proved a source of unexpected findings also in other laboratories.

Thus, Graffi and his co-workers prepared filtered extracts from Ehrlich ascites mouse carcinoma, and from several other mouse tumours. These extracts were then inoculated into newborn mice of a low leukaemic inbred strain (designated by the symbol Agnes-Bluhm and having an incidence of spontaneous leukaemia less than 1%); newborn non-inbred mice designated by symbols M and W were also injected. As a result, 34% to 75% of the inoculated mice developed a myelogenous form of leukaemia, after an average latency varying from 113 to 224 days. The most potent extracts were those prepared from Ehrlich carcinoma and from three closely related mouse sarcomas. Of 332 control mice inoculated with either normal organ extracts from mice, rats, chickens, or rabbits, or with rat-tumour filtrates, less than 1% developed leukaemia (Graffi *et al.*, 1956).

Friend inoculated cell-free centrifuged extracts from Ehrlich ascites carcinoma into newborn Swiss mice. After a latency of 14 months, some of the inoculated mice developed a form of myelogenous leukaemia. This leukaemia was found to be transmissible by intraperitoneal inoculation of filtrates into young adult Swiss mice, inducing leukaemia after only several weeks (Friend, 1957).

It is quite possible, therefore, that there exist different forms of mouse leukaemia, caused by distinct though probably related viruses, requiring different experimental conditions for cell-free transmission. Certain leukaemic agents may require newborn hosts for transmission; others may also infect adult hosts of a suitable line. In this respect, mouse leukaemia would not be different from the various forms of chicken leucosis (Furth *et al.*, 1933; Burmester *et al.*, 1946; Beard, 1957).

Electron Microscopical Studies of the Mouse Leukaemia Agent

Electron microscopical examination of the leukaemic filtrates in drop preparations, shadowed with chromium,

revealed the presence of spherical particles, varying in average diameter between 60 and 70 m μ (Gross, 1956).

More detailed studies were possible on thin sections of the leukaemic tissues. In collaboration with L. Dmochowski, sections were prepared from thymus, cervical glands, and livers from C3H mice in which leukaemia was induced by inoculation of the leukaemic filtrates. Characteristic spherical virus-like particles, most of them averaging approximately 100 m μ , and showing dense centres, were found in large numbers in the cytoplasm and in extracellular spaces (Dmochowski and Grey, 1957). Examination of normal mouse tissues did not reveal the presence of similar virus-like particles; additional studies are necessary, however, to clarify this point further. It is possible that the particles visualized in drop preparations represent the dense centres of the large particles observed on the sections. Technical differences in the manner of preparing the drop preparations as compared with ultra-thin sections may also account for the difference in size of the particles.

Multiplication of the Parotid Tumour Agent in Tissue Culture

In a series of experiments Stewart and her associates (1957) at the National Cancer Institute succeeded in demonstrating the survival and possibly also multiplication of mouse-leukaemia-derived parotid tumour agent in tissue culture media. After passing the agent through monkey kidney, or chicken chorion allantoic membrane culture media, inoculation into newborn, less than 12 hours old, (C3Hf $\times$ AKR) F₁ hybrid mice induced in up to 51% of the mice the development of parotid tumours and other neoplasms after a latency varying from 2½ to 10 months. Control mice injected with fluids from uninoculated tissue cultures failed to develop tumours. More recently Eddy and her associates (1958) at the National Cancer Institute made the remarkable observation that the mouse-leukaemia-derived agent passed through tissue culture media induced multiple malignant tumours, most frequently sarcomas, following inoculation into young, less than 4 weeks old, Syrian hamsters.

Attempt to Transmit Radiation-induced Leukaemia by Filtrates

One of the greatest difficulties in the proper interpretation of results obtained with experimental transmission of tumours and leukaemia by means of filtrates has been the fact that in many instances morphologically similar tumours could be obtained with chemical carcinogens, hormones, or ionizing radiation.

The fact that ionizing radiation, hormones, or carcinogenic chemicals may be able to induce tumours and/or leukaemia does not necessarily imply, however, that these tumour-inducing factors are similar in their pathogenic action to oncogenic viruses.

Theoretically, at least, it may be possible to assume that carcinogenic chemicals, hormones, or ionizing radiation may induce leukaemia in mice indirectly only, by activating latent viruses carried in an inactive form by apparently healthy hosts.

In order to determine whether radiation-induced leukaemia could be transmitted by filtrates, the following experiment was carried out (Gross, 1958c).

Leukaemia was induced in mice of a low-leukaemic strain (C3H)

by fractionated total-body x-ray irradiation (150 r once a week for four to six consecutive weeks). Up to 50% of the irradiated mice developed leukaemia after seven to nine months. An attempt was then made to recover from such mice an oncogenic agent; filtered extracts were prepared from livers, spleens, and thymic lymphosarcomas from the leukaemic donors; the filtrates so prepared were then inoculated into newborn C3H mice. Of 148 inoculated mice, 16 (11%) developed leukaemia after approximately one year, and 7 (5%) developed parotid gland tumours (Gross, 1958c). Similar results have been recently obtained by Kaplan (1958) on C57 Black mice. These experimental findings suggest that mice of a low leukaemic strain may carry a latent leukaemogenic agent of a low pathogenic potential. When triggered by repeated total-body x-ray irradiation such an agent may become pathogenic, and induce leukaemia in its hosts. Once activated, this agent may then, at least in some instances, be separated from cells by filtration, and transmitted by inoculation into newborn hosts of a susceptible strain.

There may exist a large variety of tumours which, although similar in their morphology, may be individually distinct. It is entirely possible that ionizing radiation, hormones, or chemical carcinogens merely activate latent oncogenic viruses; their tumour-inducing action could thus be compared to the experimental induction of bacteriophages in lysogenic bacteria (Lwoff, 1953). Accordingly, oncogenic viruses could be compared to temperate bacteriophages, transmitted from one generation to another in lysogenic bacterial hosts, only very occasionally killing their carriers (Gross, 1954; Latarjet, 1954).

Should this hypothesis prove to be correct, it would then logically follow that individuals with symptoms of tumours or leukaemia would represent only a small fraction of those actually carrying the seeds of either disease. This concept would also explain the striking though not too frequent cases of familial occurrence of tumours and leukaemia not only in animals but also in humans. Although in most instances cancer or leukaemia occurs in man only sporadically, yet numerous instances, some of them striking, have been recorded in which malignant tumours and/or leukaemia developed in two or more members of the same family, either within the same or in successive generations. Cases of either tumours or leukaemia developing in brothers, brothers and sisters, or in a child and in one of its parents, grandparents, aunts, uncles, or cousins, have been recorded by numerous observers. One of the most striking families

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).

REFERENCES

- Anderson, R. C. (1951). *Amer. J. Dis. Child.*, **81**, 313.
 Beard, J. W. (1957). *Proc. Third Nat. Cancer Conf.*, p. 336. Lippincott, Philadelphia.
 Bitner, J. I. (1936). *Science*, **84**, 162.
 Burmester, B. R., Prickett, C. O., and Belding, T. C. (1946). *Cancer Res.*, **6**, 189.
 Cluffio, G. (1907). *G. ital. Mal. Vener.*, **42**, 12.
 Cook, R. H., and Olson, C. (1951). *Amer. J. Path.*, **27**, 1087.
 DeMonbreun, W. A., and Godpasture, E. W. (1932). *Ibid.*, **8**, 43.
 — (1934). *Amer. J. Cancer*, **21**, 295.
 Dmochowski, L., and Grey, C. E. (1957). *Ann. N.Y. Acad. Sci.*, **68**, 559.
 Dulancy, A. D., Maxey, M., Schilling, M. G., and Goss, M. F. (1957). *Cancer Res.*, **17**, 809.
 Eddy, B. E., Stewart, S. E., Young, R., and Mider, G. B. (1958). *J. nat. Cancer Inst.*, **20**, 747.
 Ellermann, V., and Bang, O. (1908). *Zbl. Bakt., I. Abt. Orig.*, **46**, 595.
 Friend, C. (1957). *J. exp. Med.*, **105**, 307.
 Furth, J., and Breedis, C. (1933). *Ibid.*, **58**, 253.
 — Buffett, R. F., Banasiewicz-Rodriguez, M., and Upton, A. (1956). *Proc. Soc. exp. Biol. (N.Y.)*, **93**, 165.
 — Seibold, H. R., and Rathbone, R. R. (1933). *Amer. J. Cancer*, **19**, 521.
 Graffi, A., Bielka, H., and Fey, F. (1956). *Acta haemat. (Basel)*, **15**, 145.
 Gross, L. (1944). *Cancer Res.*, **4**, 293.
 — (1951a). *Proc. Soc. exp. Biol. (N.Y.)*, **76**, 27.
 — (1951b). *Ibid.*, **78**, 342.
 — (1953). *Ibid.*, **83**, 414.
 — (1954). *Blood*, **9**, 557.
 — (1955). *Proc. Soc. exp. Biol. (N.Y.)*, **88**, 64.
 — (1956). *Cancer*, **9**, 778.
 — (1957a). *Proc. Soc. exp. Biol. (N.Y.)*, **94**, 767.
 — (1957b). *Ann. N.Y. Acad. Sci.*, **68**, 501.
 — (1958a). *Proc. Soc. exp. Biol. (N.Y.)*, **97**, 300.
 — (1958b). *Cancer Res.*, **18**, 371.
 — (1958c). *Acta haemat. (Basel)*. In press.
 Hays, E. F., Simmons, N. S., and Beck, W. S. (1957). *Nature (Lond.)*, **180**, 1419.
 Kaplan, H. S. (1958). *Tex. Rep. Biol. Med.* In press.
 Kassel, R., and Rottino, A. (1958). *Proc. Amer. Ass. Cancer Res.*, **2**, 313.
 Kunkel, L. O. (1928). *Filterable Viruses*, p. 428. Williams and Wilkins, Baltimore.
 Latarjet, R. (1951). *Ann. Inst. Pasteur*, **81**, 389.
 — (1954). *Sang.*, **25**, 777.
 Lucke, B. (1938). *J. exp. Med.*, **68**, 457.
 Lwoff, A. (1953). *Bact. Rev.*, **17**, 269.
 — Siminovich, L., and Kjeldgaard, N. (1950). *Ann. Inst. Pasteur*, **79**, 815.
 MacDowell, E. C., and Richter, M. N. (1935). *Arch. Path. (Chicago)*, **20**, 709.
 Parsons, R. J., and Kidd, J. G. (1936). *Proc. Soc. exp. Biol. (N.Y.)*, **35**, 441.
 Pasteur, L. (1865). *C.R. Acad. Sci. (Paris)*, **61**, 506.
 Rous, P. (1911). *J. Amer. med. Ass.*, **56**, 198.
 Rudali, G., Duplan, J. F., and Latarjet, R. (1956). *C.R. Acad. Sci. (Paris)*, **242**, 837.
 Sanarelli, G. (1898). *Zbl. Bakt., I. Abt. Orig.*, **23**, 865.
 Shope, R. E. (1932). *J. exp. Med.*, **56**, 803.
 — (1933). *Ibid.*, **58**, 607.
 — (1955). *Proc. Soc. exp. Biol. (N.Y.)*, **88**, 533.
 Stewart, S. E., Eddy, B. E., Gochenour, A. M., Borgese, N. G., and Grubbs, G. E. (1957). *Virology*, **3**, 380.
 Ullmann, E. V. (1923). *Acta oto-laryng. (Stockh.)*, **5**, 317.
 Waelisch, L. (1917). *Arch. Derm. Syph. (Wien)*, **124**, 625.
 Wile, U., and Kingery, L. B. (1919). *J. Amer. med. Ass.*, **73**, 970.
 Woolley, G. W., and Small, M. C. (1956). *Cancer*, **9**, 1102.

EFFECT OF INSULIN AND GLUCAGON
ON TUMOUR GROWTH

BY

J. M. SALTER, Ph.D.

R. DE MEYER,* M.D.

AND

C. H. BEST, M.D., F.R.S.

*The Banting and Best Department of Medical Research,
University of Toronto, Toronto, Ontario, Canada*

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

*On leave of absence from Professor J. P. Hoet's Department, University of Louvain, Belgium.

†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.