a group of 10 adult hirsute women who responded well to corticotrophin. Secondly, certain patients, marked with an asterisk in Table II, may have given submaximal responses, since they received a batch of corticotrophin of poor potency by rat assay. The remainder of the patients all received very potent batches. It is therefore possible that the position of the former patients should be moved vertically upwards when compared with the remainder. In the third place, there are five adult obese males all of whom received potent corticotrophin and four responded feebly, although basal steroid excretion was very adequate. These cases will be discussed further.

Interesting results have been obtained when the ratio of the increase in ketogenic steroid excretion is compared with the increase in ketosteroid excretion (Fig. 5). For further reasons, which will become apparent, data in this chart have been confined to patients receiving a single very potent batch of corticotrophin (89754). The figures plotted represent the highest levels of ketogenic steroid or ketosteroid attained during the four days of the test. The majority of the observations fall near or below a line indicating a ratio of ketogenic steroids to ketosteroids of 2 to 1. The two normal children are below it. This indicates a relatively greater responsiveness of corticoids. Ferrazzini, Borth, and Mach (1952) have published data on 10 children, using older methods of assessment, indicating that they are very responsive in this respect, whereas the level to which ketosteroids rise is low. In one of our children, a girl aged 9, ketosteroids reached a level of 10 mg. per day, and in the other, a boy aged 11 and therefore nearing puberty, they reached 22 mg. per day. Also in this area of the chart are the patients with basal hypo-adrenal corticalism. There are nine women with post-pubertal hirsutism, all of whom have responded well to corticotrophin. Their response will be examined in greater detail.

The responses of the entire miscellaneous group of patients to corticotrophin may be summarized as follows: (1) Low ketosteroids and ketogenic steroids with no response to corticotrophin—Addison's disease. (2) Low resting steroid excretion with rapid and often high response in ketogenic steroids—basal hypo-adrenal corticalism. (3) Normal resting steroids with normal response to corticotrophin, the ketogenic steroid excretion then exceeding the ketosteroid excretion. (4) Normal to high resting ketogenic steroids, ketosteroids not usually elevated, with rapid and large response of ketogenic steroids to corticotrophin—Cushing's syndrome (see remainder of lectures, to appear next week). (5) Normal ketogenic steroids with possibly some elevation of resting ketosteroids, a good response of ketogenic steroids to corticotrophin, and a supernormal response of ketosteroids—for example, in post-pubertal hirsutism.

It is therefore meaningless to express the increase in steroid excretion due to corticotrophin as a percentage of the basal level, as has often been done in the past.

[*The conclusion of these lectures, with a list of references, will appear in our next issue.*]

In his search through documents stored in Guy's Hospital strong-room Dr. Hector C. Cameron has found information on the previously unknown ancestry of Thomas Guy, the founder of the hospital (*Guy's Hosp. Rep.*, 1956, **105**, 151). The document pertains to a meeting of Thomas and his brother, with two other members of the family, on May 6, 1687, to discharge a trust instituted by " Thomas Guy of Heckfield in the County of Southton, Clothier." He was the founder's great-grandfather. The Trust directed that £5 should be paid every feast of St. Michael the Archangel to some descendant of the same " Stock-birth." In contrast, the founder himself distributed £92,400 among people not his " Stock-birth," being chiefly the humble relatives of his mother, among whom he was brought up. Of the 50 families mentioned in his will two only had the surname Guy. The governors of the hospital paid the annuitants regularly until the last of them, Mary Hill, died in 1770.

A CLASS EXPERIMENT ON GANGLION BLOCK IN HUMAN SUBJECTS

BY

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The recent development of ganglion-blocking agents has led to their use principally in patients suffering from hypertension and a number of other disorders. Their effects in normal subjects have not been examined in the same detail, although numerous scattered observations are recorded and an attempt has been made with a group of normal subjects to correlate action with plasma concentration (Morrison and Paton, 1953). The human pharmacology class at University College provided an opportunity whereby some knowledge about the action of these drugs could be obtained in a population of healthy young adults, at the same time helping to train the students in making a number of clinical observations and in rendering these observations quantitative in a way already found valuable in a previous class (Wilson, Crockett, Exton-Smith, and Steinberg, 1950).

An experiment of this type naturally requires stringent safeguards, and in no case was a drug administered without the consent of the student. Demonstrators were always at hand to supervise the observations. Furthermore, a dose of drug and a route of administration were chosen which were known to produce only mild or moderate effects of reasonably brief duration. The class has proved successful, and has yielded valuable information on the individual variation of response to a ganglion-blocking agent. The class has now been running for four successive years, enabling observations to be made on more than 70 students, without any mishap. The development of the experimental procedure has also prompted us to devise some methods for measuring autonomic function which may be of use to others.

Methods

Systemic Blood Pressure.—This was measured, using the conventional cuff, by auscultation at the elbow, combined with palpation of the radial pulse when difficulty was found in hearing the sounds in the brachial artery. The blood pressure was taken in the supine position and then standing. To estimate the standing blood pressure sixty seconds, timed with a stop-clock, was allowed to elapse after the subject had got to his feet, and the systolic and diastolic pressures were recorded as near as possible to this time. Where postural hypotension made it impossible to obtain a reading, the subject was made to lie down despite the absence of a reading. Under these circumstances it was usually possible to state that the blood pressure was at least below a certain level.

Pulse Rate.—This was measured in the supine position and in the standing position at the same time as the blood pressure was measured, the observations being made by a second observer.

Skin Temperature.—This was measured using a thermocouple and galvanometer, with a reference junction in ice. It was usual to place a thermocouple on the plantar surface of the right big toe, marking the region with a spot of ink

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so that the same place could be used each time. The thermo-couple unit used was that devised by Professor F. R. Winton ; it consisted of a copper–constantan junction mounted on a coil of two wires enclosed in a glass tube of 8 mm. internal diameter. When the end of the tube was applied to the skin the thermocouple was held lightly in contact with the skin and shielded by the surrounding tube from draughts. The reading was taken when a steady value was obtained after about thirty to sixty seconds. The *body temperature* was also recorded, using a clinical thermometer under the subject's tongue and taking the reading after three minutes. These measurements were not made systematically during the first year, since it was not then appreciated how great a fall in body temperature could occur under the conditions of the experiments. The room temperature was also recorded.

Salivary Secretion.—As a rough measure of the salivary secretion by the subject the following procedure was used. While in the supine position he was instructed to swallow any saliva already in his mouth. He was then fed eight half-circles of filter paper (12.5 cm. in diameter), one at a time, which had been impregnated with 2.5% citric acid. The subject moved these about gently in his mouth so as to absorb the saliva formed. Sixty seconds after the administra-tion of the first piece of paper the pieces were collected from the mouth and rapidly weighed. The differences in weight between the dry filter paper and the paper collected from the subject's mouth represented the saliva secreted in one minute in response to this moderately acid stimulus.

Ocular Accommodation.—The near point and the far point were determined on the supine subject, using the con-ventional metre rule carrying a movable object. To measure the far point a 2-dioptre lens was inserted in the holder in front of the right eye so as to bring the normal far point to a distance of 50 cm. The readings were taken by moving the object-carrier towards the subject until the printing on it just became blurred and the distance of the object from the eye was noted. The carrier was then moved nearer still and then away again to find the point at which the object just became clear again. The mean of these two distances was taken as the near or the far point and then converted into dioptres.

Reaction to Light.—As it was impracticable to obtain constant conditions of illumination, a cylinder of aluminium sheet was devised which fitted at one end over the face of the subject well enough to exclude light at the junction of cylinder and face ; at the other end it fitted over the ob-server's face, enabling him to inspect the subject's pupils. A 15-watt light bulb was fitted half-way between the two, shielded from the observer ; it was arranged that this could run normally at 230 volts to give a bright light or (by intro-ducing a similar concealed bulb in series) at 115 volts to give a dim light, the change being made by a switch below the main cylinder. The size of the subject's right pupil was measured by means of a " perspex " scale on the side of which was cut a series of semicircles of diameters ranging from 5/64 to 24/64 in. (2 to 9.5 mm.) ; it was arranged so as to slide through the cylinder at a position close enough
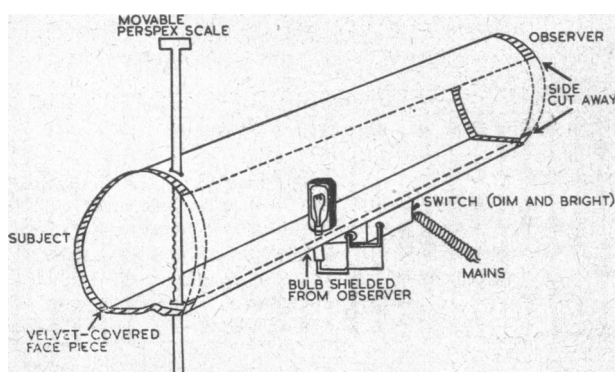

FIG. 1.—Diagram of apparatus used for measuring size of pupil.

to the pupil to enable the diameter of the pupil and that of the semicircles to be compared (Fig. 1).

Sweat Secretion.—The difficulty in measuring sweat secre-tion was that there was a steady diminution in sweating rate during the progress of the experiment. With methods such as the starch and iodine procedure it was usually found that no worth-while record of sweating was obtained after the first few periods of observation. The method devised by Sutarman and Thomson (1952), in which a plastic imprint was taken of the sweat glands, proved somewhat more satisfactory. This allows one to see the number of sweat ducts open and their size, but it is difficult to quanti-tate. Three records obtained by this technique from one subject are shown in Fig. 2. For the purpose of analysis these results have been regarded as qualitative.
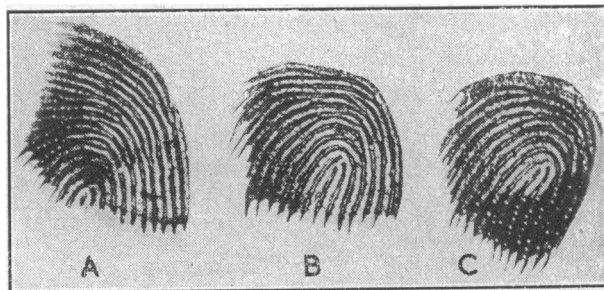

FIG. 2.—Sweat prints taken from one subject by the method of Sutarman and Thomson (1952). A : Before injection. B : Forty minutes after 25 mg. of hexamethonium. C : Sixty minutes after hexamethonium, when sweating had returned. Openings of sweat ducts are the circular clear spaces on the skin ridges.

Plan of Experiment

The students worked in groups of four, with one student as subject and the remaining three as observers dividing the various duties among themselves. A set of observations was made every twenty minutes, timed with a stop-clock, in the following order : supine blood pressure and pulse rate ; standing blood pressure and pulse rate ; skin temperature, body temperature, and room temperature ; salivary secretion and sweating rate ; ocular accommodation and reaction to light. This sequence was strictly followed, so that the interval between successive particular types of observation was kept constant. Two sets of control observations were made before injection of the drug, the subject having been instructed to take a light lunch, not to drink too much, and to empty his bladder, since he would be confined to his couch for about three hours. Immediately after the second set of control observations, 25 mg. of hexamethonium bromide was injected subcutaneously into the deltoid area by one of the demonstrators ; in some of the later experi-ments the students themselves, under supervision, made the injections.

These observations were continued for six observation periods, giving a total period after administration of the drug of two hours. By this time postural hypotension had in almost every case subsided. Occasionally, after this time, if the student stood still for as long as five or ten minutes he felt a little faint ; if this happened he was made to sit down and remain behind until he had completely recovered. As a further safeguard subjects were always asked to arrange beforehand that a colleague could see them home that evening if necessary. They were also asked to fill in the following day a report sheet on their sensations and com-ments on the experiment.

Pooled Average Results

Fig. 3 gives the magnitudes of the average effects of hexa-methonium on the supine and standing blood pressure and the pulse rate, on the body temperature, on the temperature of the skin of the big toe, on salivary secretion, on the near and far points, on the size of the pupil in dim light, and on its reaction to bright light. Although these averages
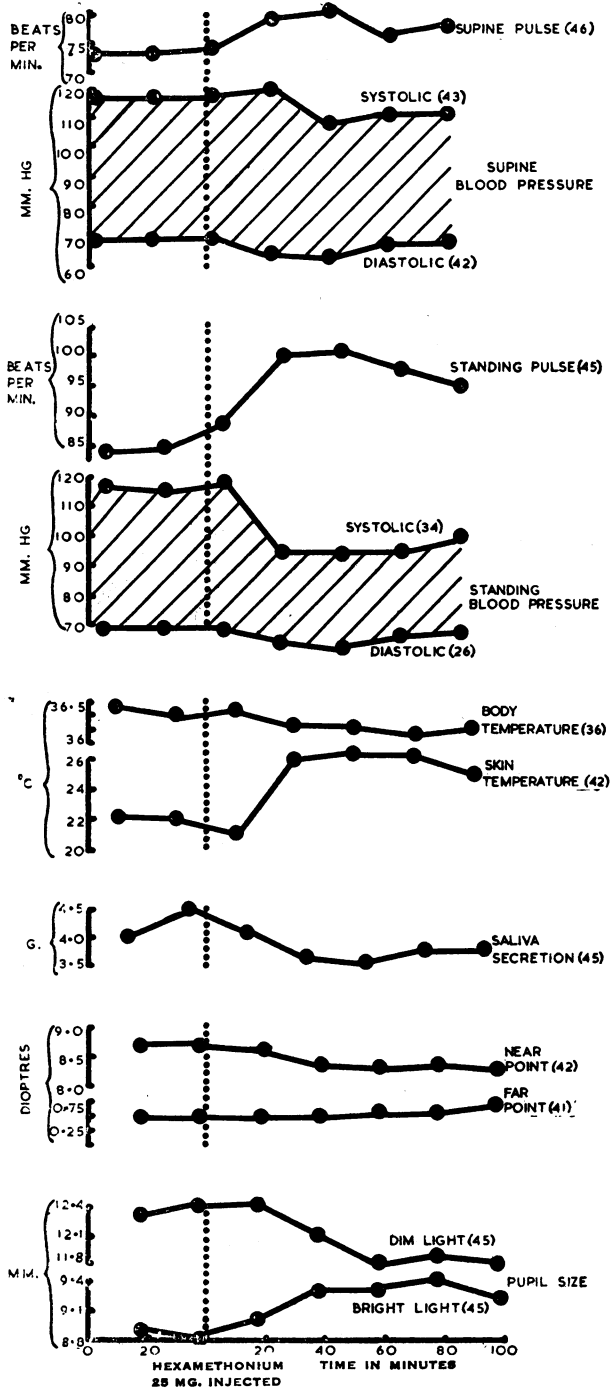
FIG. 3.—Graphs of the average effect of hexamethonium. The number of subjects for which satisfactory records were obtainable and from which the curves were constructed is given in parentheses for each test.

conceal large individual variations presently to be discussed, they serve to provide a fair picture of the predominant effects of the drug and of the time course of action. The most prominent effects are a fall in the blood pressure on standing, together with a considerable increase in standing pulse rate, an increase in the temperature of the big toe, and a fall in body temperature. In addition, there is a distinct reduction in the ability of the eye to accommodate for near vision and to react to light. There is also a detectable acceleration of the heart in a supine position and a small constriction of the pupil under dim illumination. The effect on salivary secretion is small, partly because there was a steady fall in the rate of spontaneous secretion as the experiment progressed and the time from the last meal increased.

Time of Onset of Effects

It is notable that, whereas the fall in standing blood pressure reached its peak something like forty minutes after the injection of the drug, and the rise in skin temperature a peak at about the same time or possibly a little earlier, yet the visual effects had a distinctly greater latency, usually being maximal at about sixty minutes and sometimes persisting after the cardiovascular effects had ceased. The standing systolic blood had usually returned at the end of 100 to 120 minutes to levels high enough to avoid faintness or any other symptom at the end of the one minute's standing, but might not quite have reached normal levels. The earliest sign of the action of the drug to make its appearance was usually the change in standing pulse rate, which reached a peak approximately twenty minutes after the injection.

Relative Incidence of Effects

Different subjects varied greatly in their responses to the injection of hexamethonium. Not only did the magnitude and time course of a particular effect vary from subject to subject, but also the distribution of effects on different bodily functions. Thus one subject might display pronounced postural hypotension, with no ocular signs, whereas another might have a great reduction of accommodation to light or near vision with hardly any cardiovascular effect. These differences in response have the result that the mean curves already given in Fig. 3 are seriously flattened. Fig. 4 illustrates the variation in maximum effect on the standing blood pressure; no averaged curve can satisfactorily convey the fact that in many individuals postural hypoten-
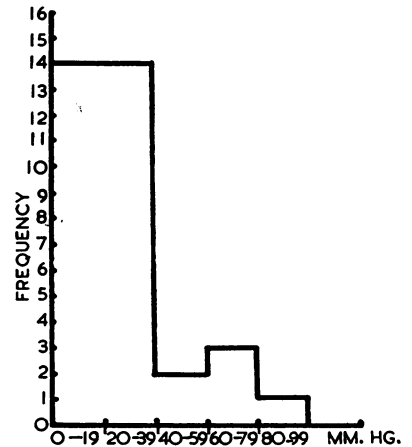
FIG. 4.—Graph of the variation in incidence of maximum fall in systolic blood pressure on standing for one minute after hexamethonium. (N=34.)

NUMBER OF SUBJECTS

FIG. 5.—Diagram of the incidence of a significant effect by hexamethonium on nine different autonomic functions in 41 subjects. (●=significant effect.)

sion is slight, whereas in others it may lead to a fall of 80–100 mm. We have therefore attempted an analysis of the variability of the responses.

To do this, we first set arbitrary criteria for the presence or absence of a given ganglionic effect, and determined the incidence of each effect for each subject (Fig. 5). These criteria, chosen bearing in mind the average results, the accuracy of the measurements, and the size of a physiologically significant change, are shown in the Table. Some subjects, for whom complete records were not obtained, have been omitted. The results for diastolic blood pressure and for far point have not been included.

Incidence of Ganglionic Blocking Actions

Physiological Sign	Criterion for Effect	% Incidence (Out of 41)
Fall in body temperature ..	0·2° C. or more	90·2
Rise in skin temperature ..	2° C. ,, ,,	75·6
Reduction in supine systolic blood pressure	10 mm. or more	68·3
Reduction in standing systolic blood pressure	20 ,, ,, ,,	65·9
Increase in supine pulse rate ..	10 beats a minute or more	48·8
Reduction in salivary secretion ..	1 g. or more	48·8
Increase in standing pulse rate ..	20 beats a minute or more	46·3
Reduction in reaction to light ..	0·8 mm. or more; or absolute reaction to light after hexamethonium less than 0·4 mm.	39·0
Reduction in accommodation at near point	1 dioptre or more	36·6

The Table shows that the commonest effects are on the cardiovascular system, or result from such an action—for example, loss of heat from the body, through vasodilatation. But even the least common action, that of reducing accommodation, was seen in over one-third of the subjects.

A point of particular interest was whether there was any pattern (in Fig. 5) in the distribution of block among different ganglia. To analyse this, comparisons were made of the simultaneous presence or absence of pairs of physiological effects, yielding a series of 2 × 2 tables to which the χ^2 test was applied, using Yates's correction. These indicated that some cardiovascular effects tend to be well correlated; thus the effects on supine and standing blood pressure and the effects on supine and standing pulse are associated. But, apart from these, there was no consistent association or dissociation of the various autonomic responses.

The failure to demonstrate any association extended even to two functions involving a single ganglion, the ciliary ganglion. Thus, in 14 subjects the reaction to light was significantly depressed, but not that to accommodation. If one compares this with the Argyll Robertson pupil, ignoring the other features of such a pupil, one could characterize hexamethonium occasionally as a "syphilomimetic" drug.

These comparisons have not enabled us to support the well-known concepts of "vagotonia" and "sympatheticotonia," so far as they imply that in particular individuals the activity of the sympathetic as a whole, or the parasympathetic as a whole, may predominate. This does not exclude the possibility, however, that autonomic activity in particular individuals may sometimes concentrate itself down particular pathways. Indeed, it was remarkable to find, when Fig. 5 had been constructed, that the distribution of ganglionic action by hexamethonium was different in each of the 41 subjects. If one assumes that those ganglionic functions which were blocked were those most active in a given subject, it seems possible that the pattern of autonomic activity may be individual to each person. We have not retested any of our subjects; but, if such tests proved reproducible, it would then be possible to assign to each individual a characteristic "autonomic fingerprint."

Special Observations

Two other observations of interest were made. The first was that when hexamethonium caused a substantial rise in skin temperature and fall in body temperature the paradoxical situation arose of a subject with warm feet who was shivering vigorously; as the vasodilatation of the legs passed off and the body temperature rose the shivering ceased. This provides evidence from an unexpected quarter that shivering may be controlled more by the temperature of the blood than by the temperature sensations of the skin.

It was also noted that not long after the hexamethonium was injected the subjects became more relaxed, almost sleepy. Those who volunteered were often the most energetic members of their groups and usually tried to direct operations even from their couch. There was a striking tendency for this bossiness to dwindle or even to be replaced by a gentle doze so far as the making of observations permitted. As the effects of the drug wore off, the subject's normal character reasserted itself. The basis for this action is obscure. There is no reason to postulate a narcotic effect for the drug. Possible explanations are the cutaneous vasodilatation or the reduction, by an action at the cholinergic nerve endings of the small motor nerve fibres innervating the muscle spindles, of the proprioceptive inflow to the brain.

The students' reports on their sensations and after-effects yielded little information of physiological interest. Many reported no after-effects at all. Others complained of tiredness, mental dulling, feeling cold, headache, dizziness, an unlocated thoracic pain, bags under the eyes, diarrhoea or constipation, sleeplessness or sleeping very well, irritability or feelings of calmness, loss of appetite or increased thirst, and loss of prowess at sport (missing putts on the 18th green; "Old Elthamians 13, U.C.H. 8").

Discussion

The observations described, giving hexamethonium to a group of 50 young students, correspond well with clinical experience of the drug. One of our purposes was to examine whether the response to the drug showed the same variations in a fairly homogeneous group as are seen clinically, and whether any pattern in the incidence of its effects could be delineated. But the variability was well marked even in this relatively restricted group; the ganglionic systems studied (sympathetic vasomotor ganglia, vagal ganglia to the heart, ciliary ganglion, parasympathetic ganglia to salivary glands, and the ganglia supplying the sweat glands) were all capable of being affected independently of each other. It appears that for the present such variation in intensity and in character of effect must be accepted.

As a teaching method the class offered a number of advantages. First, it introduced the students to methods they would use in their clinical work, and stressed the necessity of making their observations quantitative, even when using simple apparatus. Secondly, it provided a valuable supplement to their teaching in autonomic physiology and pharmacology. The discussion of the results with the class after the experiment was over often served to clear up some misconceptions. Thirdly, the experiment introduced the students to a therapeutic agent which they would later encounter in the wards and showed them some of its effects and side actions.

Summary

The results of a class experiment on ganglionic block are described, in which the effect of 25 mg. of hexamethonium on healthy student volunteers, judged by ten autonomic tests, was studied.

The most common effect was a fall in body temperature (90% incidence) and rise in skin temperature (76%); and then (in descending order of frequency), fall in supine and standing systolic blood pressure, rise in supine and standing pulse rate and reduction in salivary secretion, and reduction in the reaction of the pupil to

light and to accommodation (30–40% incidence). Sweating was also reduced.

The incidence of ganglionic block over the various autonomic functions tested was different in every one of the 41 subjects for which complete records were obtained.

Variability in the response to a ganglion-blocking agent is as great in normal subjects as in patients. It may be regarded as a result of the varying autonomic constitution of different individuals.

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TREATMENT OF MURINE LEUKAEMIA WITH X RAYS AND HOMOLOGOUS BONE MARROW

PRELIMINARY COMMUNICATION

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When mice are given an otherwise lethal dose of x rays to the whole body they can recover if injected intravenously with homologous myeloid cells. It is now established that their depleted haemopoietic and lymphopoietic tissues are colonized by cells derived from those which have been injected (Ford et al., 1956). This suggests that leukaemia of the mouse might be successfully treated. On the one hand, the dose of x rays which is sufficiently lethal to normal cells of the bone marrow and lymphatic tissues to cause death of the animal might well be completely lethal to leukaemic cells : the irradiated animal could then be treated with normal isologous bone marrow from the same strain of mouse for the repopulation of the haemopoietic and lymphopoietic tissues. On the other hand, if the dose of x rays sufficient to kill the animal is not 100% lethal to leukaemic cells, the malignant condition would in these circumstances recur by growth from the surviving cells, since neither host nor graft has the ability to resist ; but, if homologous bone marrow from a different strain of mouse were given, the colonizing cells might retain the capacity of the donor to destroy by the reaction of immunity these residual leukaemic cells—and perhaps also the host.

This preliminary communication deals with the results of three similar experiments designed to test the former hypothesis.

Experimental

Animals.—Mice of the CBA/H strain, inbred by strict sib-mating for many generations with frequent re-selection of sublines, were the test animals. They were three to four months old at the time of test. Mice of the same strain were used as isologous donors. Mice of an equally highly inbred A/H strain and hybrid mice, $T_6/+$ (Carter et al., 1955), were homologous donors.

Tumour.—The leukaemia used in these experiments and nominated 151/1 was induced by means of chronic irradiation in a CBA mouse by our colleague, R. H. Mole. It can be passaged as a cell-suspension of spleen, lymph node, etc., to 100% of our CBA/H mice by intravenous and intra-peritoneal injections of 10^6 cells. The response following subcutaneous injection is nearly 100%, but an occasional animal is resistant. When it has been given to the inbred strains, C_3H/H, A/H, C57/H, and 101/H, and to various types of hybrid mice using the same doses and routes, there have been no instances of leukaemia. In the CBA/H mouse it produces a generalized replacement of lymphoid tissue and bone marrow and an interstitial invasion of most other tissues with lymphoid cells resembling large lymphocytes. The leukaemia is relatively aleukaemic, the leucocyte count of peripheral blood being usually 20,000 to 50,000 per c.mm. Death from leukaemia in most cases occurs within one month of injection and certainly within two months in those animals in which the leukaemia has " taken." Survival for a period of three months has therefore been taken as test of cure.

As a routine the CBA/H mice were given 10^6 leukaemic cells in suspension in physiological saline containing 0.3% sodium citrate and were irradiated one week later.

X-radiation.—Acute doses were given in 14 minutes: the subacute doses were spread over 25 hours. In each case the quality of radiation was the same. The tube potential was 250 kV constant potential ; H.V.L., 1.2 mm. Cu.

Results

For normal CBA/H mice given the x-irradiation in 14 minutes the LD_{98} is 950 rad.‡ The LD_{98} is 1,340 rad when the dose is spread over 25 hours.

CBA/H mice injected intravenously one week previously with 10^6 leukaemic cells, and then acutely irradiated with a dose of 950 rad and treated with isologous bone marrow or infant spleen, died after about a month with generalized leukaemia. (Mean survival of a group of 10 treated mice was 31.6 days (S.D. 8.0 days) from the time of irradiation, compared with 8.5 days (S.D. 2.6 days) of 10 unirradiated control mice.)

On the other hand, CBA/H mice injected one week previously with 10^6 leukaemic cells, irradiated over 25 hours to a dose of 1,500 rad, and then treated with bone marrow or infant spleen have shown survival of three months and longer in three successive experiments (see Table).

Animals Surviving (A) and Dying (†) After Treatment With Subacute Whole-body X-irradiation and Intravenous Bone Marrow

Experiment:	1	2		3
10^6 leukaemic cells given (date)	S.C. (14/3/56)	S.C. (26/4/56)	I.V.	S.C. (24/5/56)
Unirradiated controls	† † † † †	† † A A	† † † †	† † † † †
Experimental— (date) 1,500 rad x rays followed by bone marrow I.V. from mice: CBA/H ..	(21–22/3/56) A A A A A A A A A †	(3–4/5/56) A A A A A	A A A † † *	(31/5–1/6/56) A A A † †
A/H ..	—	—	—	A A †* †* †* A A A †* †*
$T_6/+$ ..	—	—	—	

S.C. = Subcutaneously. I.V. = Intravenously.
* Deaths not attributable to leukaemia.

In experiment 1, in which the leukaemia was induced by subcutaneous injection, 9 out of 10 treated mice have survived for over five months ; all are in good general condition and are apparently normal apart from grey hair. The tenth

‡The doses are recorded as rads estimated in soft tissue.