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RUNT DISEASE*

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Runt disease is an entity restricted to laboratory animals, and though it has no known counterpart in clinical medicine it promises to contribute to the better understanding of a variety of problems in man. The condition arises when immunologically competent cells are injected into a homologous host which for one reason or another is unable to reject them. (The term "homologous" in this context refers to an animal of the same species as the donor, but of different genetic constitution.)

Since the original cellular injection is a homograft, the sickness of the host is believed to be due to a graft-versus-host reaction. Non-reactivity of the host against the graft occurs in the following situations:

(1) The host is immunologically immature at the time of receiving the foreign cells and develops tolerance rather than immunity—that is, it comes to regard the foreign cells as "self," and accordingly does not react against them (Billingham *et al.*, 1953, 1956).

(2) The host is immunologically mature, but contains all the antigens present in the graft, which is accordingly regarded as "self." The graft, however, does not contain all the antigens present in the host and can recognize the host cells as foreign. Such a situation occurs when an F1 hybrid (AB) of two inbred parental strains (A and B) receives mature immunologically competent cells from either A or B. The hybrid AB will accept either A or B as "self," but neither A nor B will recognize AB as "self." The grafted parental-strain cells may thus bring about a graft-versus-host reaction in the hybrid recipient (Boyse, 1959; Gorer and Boyse, 1959a; Kaplan and Rosston, 1959; Trentin, 1959; and others).

(3) It is possible, though by no means proven, that a large enough dose of homologous cells may completely overwhelm the immunological defences of the mature host, so leaving the grafted cells free to react against the host (Castermans, 1958).

The term runt disease was originally used to refer to the illness sometimes complicating the first of the three experimental situations described above (Billingham and Brent, 1957). Since then, however, it has also come to be used for the sickness occurring when F1 hybrid hosts receive parental-strain cells (Oliner *et al.*, 1961).

In the ordinary course of events a syndrome confined to laboratory animals and without a counterpart in man could not be expected to arouse widespread interest, especially if its mode of production were not related to

any procedure commonly employed in clinical medicine. In the case of runt disease, however, there are several reasons why a problem of this nature may be of importance in the realms of clinical medicine:

(1) The existence of runt disease largely explains why the recent laboratory advances in homografting have not been applicable to man. For instance, it is now relatively easy to induce some laboratory animals to accept skin homografts for long periods of time (Billingham *et al.*, 1953). This is not yet possible in man.

(2) When animals are lethally or sub-lethally irradiated and subsequently transfused with homologous marrow the so-called secondary disease or homologous disease which ensues is almost certainly analogous to runt disease. (Reviewed by Barnes *et al.* (1957) and by Van Bekkum *et al.* (1959).) Potential graft-versus-host reactions can thus be expected to limit the treatment, for instance, of leukaemia and malignant disease by heavy doses of radiation or anti-mitotic drugs in combination with homologous marrow transfusion (Woodruff, 1961). The treatment of severe radiation damage by this method will likewise be potentially hazardous.

(3) Runt disease is in many ways equivalent to, though by no means identical with, the autoimmune situation in man (Kaplan and Smithers, 1959; Oliner *et al.*, 1961) in so far as immunologically competent cells regarded by the host as "self" are able to react against other host body components.

(4) While this is not a new experimental tool (Danchakoff, 1916; Murphy, 1916), its significance as a possible approach to a variety of problems has been appreciated only recently. For example, Burnet and Burnet (1960) have used the graft-versus-host reaction as a method of elucidating certain aspects of the clonal selection theory of acquired immunity. Oliner *et al.* (1961) have likewise related the haematological abnormalities seen in runt disease to those regarded as being autoimmune in man. If the suggestions of Billingham and Brent (1959) and Billingham and Silvers (1960) are correct, graft-versus-host reactions may also be of importance in understanding some of the hitherto unexplained aspects of foetal, neonatal, and obstetrical pathology.

Events Leading up to Recognition of Runt Disease

For nearly 50 years it has been realized that the transplantation of some adult tissues to the chorio-allantoic membrane of developing chicks may be accompanied by splenomegaly in the host in association with characteristic pathological lesions (Danchakoff, 1916; Murphy, 1916). It would now appear that these represented some of the earliest recorded examples of graft-versus-host reactions. The idea that a homograft

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might be able to react against its host was suggested by Dempster (1953) and Simonsen (1953) in the light of histological changes seen in homotransplanted kidneys. The latter were infiltrated with plasma cells and their precursors, which were assumed to have arisen in the graft and to represent an immunological reaction on the part of the graft against its host. Subsequent experiments have cast considerable doubt on the validity of this interpretation (Mannick *et al.*, 1959; Fowler and West, 1960; Murray *et al.*, 1960; Porter and Calne, 1960; Zukoski *et al.*, 1960). However, the idea proved a fruitful one, and in 1957 it was elaborated by Billingham and Brent to explain the mortality consequent upon the induction of immunological tolerance in certain donor-host combinations in inbred mice. They originally used the term runt disease on purely descriptive grounds. At about this time Woodruff and Sparrow (1957) noted the occurrence of a similar disease entity in rats, and Simonsen (1957) described a comparable condition in young chickens receiving homologous adult cells.

Immunological Tolerance

Medawar (1961) describes immunological tolerance as "a state of indifference or non-reactivity towards a substance that would normally be expected to excite an immunological response." Tolerance as a naturally occurring phenomenon was first recorded by Owen (1945) in dizygotic (genetically non-identical) twin cattle. Because of vascular anastomoses between the placentas, foetal twin cattle exchange not only mature blood-cells but also haemopoietic cells, whose mitotic progeny persist into adult life. Each twin thus comes to possess two genetically different types of circulating blood-cell. In these circumstances the host accepts foreign blood-cells which would otherwise have stimulated an immune reaction. The significance of this finding was apparent to Burnet and Fenner (1949), who postulated that, "if in embryonic life expendable cells from a genetically distinct race are implanted and established, no antibody response should develop against the foreign cell antigens when the animal takes on an independent existence." The proof of this postulate was supplied by Billingham *et al.* (1953, 1956), who were able to show that if embryonic or neonatal mice were confronted with nucleated homologous cells they were unable to react immunologically against that donor in adult life.

There is reason to believe that the transplantation antigens of a given individual are common to all the nucleated cells (Medawar, 1959a; Hicken and Krohn, 1960). Accordingly the tolerance extended to the transplantation antigens present in the original inoculum will also be extended to the other tissues of that donor, but not to the tissues of a genetically dissimilar animal. Thus a skin homograft will be accepted by an animal made tolerant by the injection of homologous lymphoid cells as in theory should any other tissue from the donor in question.

While many problems relevant to the nature of immunological tolerance still remain to be elucidated, the ease with which tolerance of homologous transplantation antigens can be produced experimentally in suitable strain combinations has made available much otherwise inaccessible information in the field of tissue transplantation. In addition, as Eichwald and Lustgraaf

(1958) have commented, the study of immunological tolerance is likely to prove at least as fruitful in other fields of endeavour.

Runt disease has been a complication of the experimental induction of tolerance using immunologically competent cells. While it is by no means essential to use an inoculum containing immunologically competent cells, nevertheless such tissues as spleen, lymph nodes, marrow, and thymus have two outstanding advantages: (1) they are easily obtained and made into suspensions for injection; and (2) their component cells are known to "home" readily to the lymphoid tissues of the host, where they are presumed to exert their tolerance-conferring properties (Mitchison, 1956).

Is Tolerance a Necessary Prerequisite for Runt Disease?

If runt disease is regarded as the outcome of an immunological attack on the host by the graft, then it follows that such an attack is possible only if the grafted cells survive in their new host—that is, the host is tolerant of the graft (Medawar, 1959a). While agreeing that this is probably true for the majority of experimental situations so far studied, Billingham and Brent (1959) suggest that it may not invariably be so. They base their argument on the fact that in one strain combination in mice runt disease is common following neonatal injection of spleen cells, though tolerance never occurs. On the other hand, the mortality can be circumvented if bone-marrow cells are used instead of spleen cells, though the resultant tolerance is weak. They argue that, since tolerance is never produced in this combination after the neonatal injection of spleen cells, it seems probable that the runts resulting from this procedure may well be non-tolerant. They suggest that the donor cells may react with such vigour that the host is killed before its immunological defences can be alerted.

A satisfactory solution to this problem will not be attained until more is known about (1) the exact dosage of cells necessary for the production of tolerance in a given situation; and (2) the number of immunologically competent cells necessary for the production of runt disease in the same situation. Comparison of the effects of similar dosages of marrow cells and spleen cells is difficult on account of variations in (1) the numbers of cells which are immunologically competent; (2) the number of cells which are capable of mitotic division within the new host; (3) the normal survival times of the different component cells; and (4) the capacity of the component cells for "homing" (Mitchison, 1956) to immunologically reactive centres.

Production of Runt Disease

In the following account results from various experimental studies, using several species of animal, are compared. They are also considered in the light of our own series of 89 rats with runt disease. The latter series was obtained not by design but in the course of producing immunological tolerance for the purposes of other experiments. Most of the rats noticed to be suffering from runt disease were killed when moribund and subjected to post-mortem examination. Tissues were fixed in formol saline and stained routinely with haematoxylin and eosin and pyronin-methyl green.

Selected sections were stained with Marshall's (1956) modification of the Weil-Davenport stain, Perl's prussian blue, toluidine blue, and haematoxylin counterstained with van Gieson. Haematological examination was carried out immediately before death in selected animals, and in the latter half of the series a weight-gain assay was undertaken in the manner described below.

While there is an extensive literature dealing with homologous or secondary disease in radiation chimeras (reviewed by Barnes *et al.* (1957) and by Van Bekkum *et al.* (1959)), a situation almost certainly analogous to runt disease, the present description has been almost entirely restricted to runt disease as it is seen in non-irradiated hosts.

Nature of Homologous Inoculum

While it is generally agreed that the inoculum must contain living immunologically competent cells (Siskind and Thomas, 1959) from a mature donor (Kaplan and Rosston, 1959) if it is to produce runt disease, the exact nature of the cell or cells responsible remains in doubt. There is increasing evidence for the incrimination of the lymphocyte in this respect (Terasaki, 1959; Anderson *et al.*, 1960; Burnet and Burnet, 1960), and, since the small lymphocyte is believed to be incapable of division (Gowans, 1957), circumstantial evidence points to the large lymphocyte as the cell responsible for graft-versus-host reactions. This being so, it is not surprising that cell suspensions which contain a high proportion of lymphocytes—for example, from the thoracic duct, lymph nodes, and spleen—should be more potent producers of runt disease than inocula containing relatively few lymphocytes, such as bone-marrow (Billingham and Brent, 1959; Billingham *et al.*, 1960). The position of thymic cells is less clearly defined. Billingham and Brent (1959) report runt disease in mice following the injection of thymocytes. On the other hand, Terasaki (1959) and Gorer and Boyse (1959b) were unable to produce evidence of a graft-versus-host reaction following the injection of homologous thymocytes into embryo chicks and F1 hybrid mice respectively. Kaplan and Rosston (1959) report that thymocytes are less effective than spleen cells in producing runt disease. Cole and Ellis (1958) and Vos *et al.* (1959) also note that in irradiated mice spleen cells and lymph-node cells are much more effective than thymocytes in producing secondary disease. It is possible that thymic inocula may sometimes contain tissue from the parathymic lymph nodes, which are often closely associated with the thymus, in which case the interpretation of results using these cells is rather more complicated.

Cell Dosage

Varying doses of cells have been reported as producing the stigmata of runt disease, ranging from 30,000 lymphocytes in chicks (Terasaki, 1959) and 50,000 spleen cells in newborn mice (Russell, 1960) to 125 million spleen cells per gramme of body weight in adult mice (Castermans, 1958). It is obviously difficult to compare the results of different dosages when there is variation in the species, strain combination, cellular inoculum, and age of recipient. By and large, however, it seems that the incidence of runt disease for a given strain combination increases as the dose increases (Siskind and Thomas, 1959). The time interval between inoculation

and development of symptoms is said not to vary with dosage (Russell, 1960), though Oliner *et al.* (1961), using F1 hybrid hosts, note an inverse relationship between cell dosage and the length of this latent period. Cole and Ellis (1958) record earlier deaths with higher doses, though no comment is made on the time of onset of symptoms. In our own experience the incidence of runting increased as the dosage of spleen cells became higher, as did the severity of the disease. There was likewise a noticeable increase in the early deaths during the first week with the higher cell doses. As the spleen-cell injections were invariably given subcutaneously or intraperitoneally there was little reason to ascribe this to embolic phenomena. The early deaths are usually explained as resulting from the effects of toxic cytoplasmic debris (Gorer and Boyse, 1959a; Billingham and Brent, 1957, 1959), particularly when the cells are injected intravenously. Evidence from other branches of this field (Hildemann, 1956; Burnet and Burnet, 1960; Zeiss *et al.*, 1960) indicates that the homograft reaction can manifest itself within a matter of hours. For this reason the suggestion that the early deaths following homologous-cell inoculation are entirely non-immunological seems open to doubt.

Time of Inoculation

In both F1 hybrids receiving parental-strain cells (Trentin, 1959; Howard, 1961; Oliner *et al.*, 1961) and animals receiving neonatal homologous-cell injections (Billingham and Brent, 1959; Siskind and Thomas, 1959) the younger hosts tend to show a higher incidence of runting. Simonsen and Jensen (1959) likewise noted more consistent hepatomegaly (regarded by them as indicating a graft-versus-host reaction) in young F1 hybrid hosts than in older animals receiving parental-strain spleen cells. In the case of newly born hosts the incidence of runting is probably largely bound up with the tolerance-responsive period—that is, the period during which the host reacts to the foreign cells by becoming tolerant—for the species and strain in question. In the case of F1 hybrid hosts the reason for this result is less clear.

Billingham and Brent (1959) were unable to produce runting with intravenous homologous spleen cells in mice 4 days old in one strain combination. Siskind and Thomas (1959), on the other hand, report a diminishing incidence of runt disease up till the sixth day of life in mice. The occurrence of runting at 5 days is possibly of as much significance as the fact that the incidence decreases with time after birth, considering the short tolerance-responsive period in mice (Billingham *et al.*, 1956). In view of the results of Castermans (1958), who was able to produce a syndrome apparently similar to runt disease in normal adult mice if a large enough homologous inoculum were used, it would be interesting to observe the effects of graded increases in the dose of homologous cells paralleling the weight of the recipient. It has already been suggested (Billingham *et al.*, 1960) that the long tolerance-responsive period reported by Woodruff and Simpson (1955) in rats may have been due to the fact that they increased their dosage of cells in older animals. If the results of Woodruff and Simpson (1955) and also those of Castermans (1958) are reproducible—that is, if tolerance can be produced at any age so long as the tolerance-conferring inoculum is large enough—then the nature of the tolerance-responsive

period will require reappraisal. A detailed study of this nature has not been published to date, but could be expected to throw considerable light on the mechanism of both tolerance and runt disease.

Transplantation Genetics in Relation to Runt Disease

The antigens which are responsible for eliciting the homograft reaction are believed to be represented in all the nucleated cells of the individual (Medawar, 1959a). In inbred mice—the species in which transplantation genetics has been most extensively studied (Gorer, 1956; Snell, 1957)—the antigenic substances are determined by multiple dominant Mendelian genes. A number of different loci are involved, and, as far as can be determined from those so far studied, it appears that each locus may be tenanted by multiple alleles. The total number of histocompatibility antigens in any one individual may therefore be very high. The strength or obtrusiveness of the histocompatibility antigens varies, and it is conventional to refer to strong and weak antigens, the strength or obtrusiveness being measured—albeit crudely—by the survival time of standard skin grafts exchanged between members of inbred strains (Berrian and Jacobs, 1959). A strong or obtrusive antigen in a graft will bring about its rapid rejection so long as the host does not possess the antigen in question. Conversely, a weak antigen will take longer to bring about an effective homograft reaction in the host. While there is no reason to think that the genetics of transplantation immunity is not similar in most of the species studied so far, the work in this field has been mainly confined to mice, where not only are there a number of inbred strains but also a considerable amount of genetic information is available in respect of their histocompatibility antigenic configuration. (The use of inbred or isogenic strains of laboratory animals is almost obligatory for most experiments in the field of tissue transplantation, since this represents the only way of obtaining a sufficient number of animals of similar histocompatibility antigenic configuration.)

Antigenic differences between donor and host are of major significance in the production of runt disease. In strain combinations where the donor and host differ in respect of strong histocompatibility antigens, runt disease is much more readily produced than in the reverse situation where the genetic disparity is not so great. In fact, in some donor-host combinations—at least when using spleen cells—it is virtually impossible to produce healthy tolerance in the absence of runt disease (Billingham and Brent, 1959). From the rejection times of skin grafts exchanged between members of inbred strains it is possible to predict which strain combinations will produce a high incidence of runt disease (short survival times indicating great antigenic disparity between the strains) and which combinations should yield a high proportion of healthy tolerant animals with relatively few runts (longer skin-graft survival times indicating lesser degrees of genetic disparity between the strains). The skin-graft rejection times and the incidence of runt disease cited by Billingham and Brent (1959) in mice and by Billingham *et al.* (1960) in rats are interesting in this connexion, the incidence of runt disease showing a fairly close parallel to the skin-graft rejection time for the donor-host strain combination in question.

Protection Against Runt Disease

It is possible to avoid runt disease, or alternatively to protect the host, in a number of situations:

(1) If embryonic immunologically competent cells are used instead of adult cells they should react by becoming tolerant of the host tissues rather than immune to them (Billingham and Brent, 1959; Simonsen, 1957).

(2) The inoculum contains adult cells which are not immunologically competent, or alternatively contains relatively few immunologically competent cells. In suitable strain combinations in mice, if equivalent numbers of bone-marrow cells are substituted for spleen cells the incidence of runt disease is considerably diminished (Billingham and Brent, 1959). Likewise, the use of blood monocytes has been shown by Terasaki (1959) to prevent runting in chickens.

(3) Mature F1 hybrid cells (AB) are injected into immature parental-strain hosts (A or B). The hybrid cells are unable to react against the parental-strain host cells, since they possess all the antigens of the latter. The parental-strain hosts are, of course, insufficiently mature to react against the donor cells (Billingham and Brent, 1959).

(4) Runting may be prevented by the administration of mature immunologically competent cells isogenic (genetically identical) with the host cells at the same time as the homologous inoculum or within a short time of its administration (Siskind and Thomas, 1959; Russell, 1960). The diminution in runting achieved by this procedure has been interpreted as indicating that the host has been supplied with an adult-type reticulo-endothelial system (from the mature isogenic cells) and is therefore capable of destroying the foreign cells before they are able to damage the host cells. If the clonal selection theory of acquired immunity is correct (Burnet, 1959) an alternative explanation might be suggested. Of the total number of homologous cells only a proportion—presumably a relatively small one—should be capable of a response directed specifically against the host's histocompatibility antigens. Previous contact with cells isogenic with the host might conceivably bring about the selective destruction of homologous cells whose immunological activity was specifically directed against host antigens, without necessarily conferring "adoptive immunological maturity" on the host. This being the case, it should be possible to prevent runt disease if before injection the homologous cells were incubated with a relatively small proportion of isologous cells. Initial experiments of our own using an inoculum of spleen cells in the proportion of 10 homologous to 1 isologous have shown some diminution in the expected incidence of runt disease. These results, however, require confirmation and further elaboration, particularly with observations on the rejection times of subsequent donor-strain skin homografts.

(5) In some strains of mice Siskind and Thomas (1959) were able to reduce the incidence of runt disease by immunizing the mother against the donor strain. The exact significance of this finding is uncertain at the present time.

(6) Siskind and Thomas (1959) were able to protect against runting in certain strain combinations in mice by the administration of a specific antiserum. The significance of this finding is likely to remain obscure

until more is known about the type of immunological reaction responsible for runt disease. The results of Russell (1960) are at variance with these findings.

Clinical Features of Runt Disease

The clinical features of runt disease in mice, rats, and rabbits have been described in several publications (Billingham and Brent, 1957, 1959; Woodruff and Sparrow, 1957; Billingham *et al.*, 1960; Nisbet *et al.*, 1960a, b; Porter, 1960; Oliner *et al.*, 1961; and others). While there is general agreement on the pattern of development of the disease, individual details show some variation, which may be ascribed to differences in species, strain of animal within a given species, cell dosage, and route and time of injection, as well as less tangible differences in the housing and care of the animals, which will vary from laboratory to laboratory.

The animals usually become noticeably ill about a fortnight after the initial injection, though weight-gain assays may give earlier evidence of runt disease (Russell, 1960). Increasing the cell dosage has been said not to shorten the time interval between the injection of cells and the development of overt clinical symptoms (Russell, 1960), though neither our own results nor those of Oliner *et al.* (1961) support this contention. The animals show retarded gain in weight (in those injected neonatally), or alternatively loss of weight (F1 hybrids receiving parental-strain cells), pallor, and general failure to thrive (Fig. 1). The skin often shows oedema,

exfoliation, and is inelastic (Fig. 2), and growth of fur is retarded by comparison with controls. In older animals epilation may be evident. Most observers have described peculiarity of gait. This has been attributed by Billingham *et al.* (1960) to a loss of elasticity

FIG. 3.—Rear view of runt rat to show abnormal stance. Hind paws are drawn in under body, which causes rat to topple over easily; feet are extended, causing high-stepping gait. Normal control litter mate for comparison.

and tonicity in the skin, causing difficulty in flexing the joints. Most of our runts showed hunching, with a peculiar high-stepping gait and uncertain balance; they appeared to walk on tip-toe on a very narrow base (Fig. 3). The condition of the skin did not seem entirely to account for the gait, though subsequent post-mortem and histological examinations of joints, muscles, and central nervous system in a few animals likewise failed to explain it. It is possible that the condition of the skin is a non-specific phenomenon associated with severe systemic disease. The effect of starvation must also be taken into account, as these animals are generally too weak to feed. Diarrhoea and continual sneezing are apt to cause considerable distress, and often sores appear round the mouth and anus.

The time of death varies from series to series, but generally the majority of animals with acute disease have died within six weeks of injection. In our series 73% died during this time. On occasions the disease may run a more chronic course, and Oliner *et al.* (1961) report low-grade chronic runt disease punctuated by acute exacerbations. Milder forms occur, though it is not yet certain whether those rats which show stunted growth only have actually been runts. (Clinical problems related to the diagnosis of recovered runts and chronic runts are discussed elsewhere.) Most of the recorded series to date have concerned themselves mainly with the more acute variants of the syndrome.

Our own series of Wistar rats injected with spleen cells (Nisbet *et al.*, 1960a, b) showed a very distinctive phenomenon which, to the best of our knowledge, has not been recorded in other series of runt disease. While pallor and very occasional frank jaundice were evident in

FIG. 1.—Runt rat and healthy litter mate. Growth is retarded, the fur is unhealthy, and there are excoriations around the nose.

FIG. 2.—Two-week-old runt rat to show exfoliation of skin. Skin is thin and inelastic. Diarrhoea and perianal excoriation are visible.

a number of the animals, a significant proportion went through a phase of distinct redness of the skin. The redness was very pronounced when affected animals were contrasted with paler runts, and did not seem to be associated with dermatitis. Sometimes the animal died during the red phase, but in a few instances redness lasted a few days and finally gave way to pallor before death. In no instance was a pale runt seen to become red. From the histological and haematological features there was reason to believe that the redness was due to excessive haemopoiesis (see later). Similar redness has been described in one partner of a parabiotic pair, though the redness in these circumstances may well be due to other causes (Finerty, 1952; Eichwald *et al.*, 1960).

Weight-gain Assay in Runt Disease

Billingham and Brent (1959) and Russell (1960) by weighing mice inoculated neonatally with homologous cells have shown that in those which ultimately suffer from runt disease gain in weight ceases about five to eight days after injection. From that time on there is very little increase in weight until the time of death. Failure to gain weight has also been reported by Woodruff and Sparrow (1957), Anderson *et al.* (1960), and Billingham *et al.* (1960) in rats, by Biggs and Payne (1961a, b) in chicks, and by Porter (1960) in rabbits. Loss of weight in normal neonatal and young adult F1 hybrid mice injected with parental-strain lymphoid cells has been reported by Billingham and Brent (1959), Nowell and Cole (1959), Trentin (1959), and Oliner *et al.* (1961). Castermans (1958) recorded loss of weight in normal adult mice receiving very large doses of homologous cells. Howard (1961), however, states that loss of weight alone is not an absolute indication of a graft-versus-host reaction. In our own series weight-gain assay was carried out on a number of injected animals irrespective of whether or not they ultimately suffered from runt disease, and a number of normal controls.

Gain in Weight in Individual Rats

The average weight of the newborn inbred Wistar rat used by us is approximately 5 g. The inbred rats were fostered to outbred Wistar rats at birth in order to hasten the breeding programme, and also because the inbred Wistar females do not raise their litters very

well. Variations of 4–6 g. are found between individual members of a litter, and thus a given rat may start with a considerable physical disadvantage. The more puny rats are often poor at suckling, as is shown by the absence of milk-curd in the stomach. Normally the stomach is distended with curd, which is easily seen through the transparent abdominal wall. Neonates which fail to thrive, and most runts, have empty stomachs at necropsy, so that some of the findings in both groups must be due to starvation. Even the control rats may lose weight in the first two days, and as this finding is not seen in the unfostered litters it must presumably be attributed to the fostering. In addition to these hazards, there seems no reason why the injected immunologically competent cells should not be able to exert some effect on their new hosts within a few days of injection (Hildemann, 1956; Burnet and Burnet, 1960; Zeiss *et al.*, 1960). The failure of neonates to thrive and early death during the first week will obviously be due to a variety of factors, but the mortality is far higher in the injected litters, which would suggest that the cellular inoculum is implicated in some way.

Average Gain in Weight of Control and Injected Litters

The average weights of control litters steadily increased (Fig. 4), while the average weights of the injected litters remained stationary until the fifth or sixth day (Fig. 5). Thereafter the gain in weight of each litter remained steady, though the injected litters always lagged behind the controls—for example, at 10 days the average weight of control litters was 17 g., while that of the injected litters was 12 g. (Fig. 6). At 20 days the controls were 34 g. and the injected animals 25 g. This divergence increased during the fourth week, when stunted growth or runting became more manifest.

Further analysis by comparison between the average weights of the heaviest rats, the average litter weights, and the average weights of the lightest rats showed that, irrespective of the eventual outcome, all the rats in injected litters failed to gain weight during the first week, which would seem to suggest that some reaction begins very soon after the injection. This difference between control and injected litters persisted, so that by 20 days the average weights of the heaviest rats of the injected litters were of the same order as the average weights of the lightest rats of the uninjected litters. In time many

FIG. 4

FIG. 5

FIG. 6

FIG. 4.—Average weights of heaviest and lightest rats in four uninjected litters, compared with litter average weights. ○ Heaviest average weight; ● litter average weight; × lightest average weight. FIG. 5.—Average weights of heaviest and lightest rats in 12 injected litters compared with litter average weights. Inoculum consisted of 10 to 20 million homologous lymphoid cells. ○ Heaviest average weight; ● litter average weight; × lightest average weight. FIG. 6.—Average weights of 32 uninjected and 97 injected rats during first 23 days. Inoculum consisted of 10 to 20 million homologous lymphoid cells. ○ Normal; ● injected.

of the injected rats gained full weight, but others remained permanently stunted.

The healthy tolerant animals in the injected litters showed a gain in weight either equal to or greater than the average for the litter—that is, there was no evidence that tolerance was accompanied by any stunting of growth other than that associated with the injection of homologous cells. (In the strain combination used by us 75% of the injected animals were either non-tolerant or partially tolerant; 10% were highly tolerant; 15% developed runt disease.)

Effects of Cell Dosage

(1) *High Cell Dosage (20–30 Million Cells).*—After a slight increase in weight at the end of the first week a steady fall occurred until death ensued within two or three weeks. The majority of animals in this group succumbed early in this manner if they survived the first few days. This group showed a high incidence of early deaths during the first week.

(2) *Medium Cell Dosage (10–20 Million Cells).*—Fewer animals developed runt disease, and those which did tended to lose weight over a longer period. The number of early deaths was considerably less than in the group receiving high cell dosage.

(3) *Low Cell Dosage (Below 10 Million Cells).*—The incidence of early deaths was virtually eliminated. There was no overt runting, and the only abnormality was retarded gain in weight of the pattern illustrated in Fig. 6.

It is therefore evident from this series that, though reduction in the dosage of cells eliminates runting, a systemic effect is still apparent, as revealed by the weight-gain assay.

(To be concluded, with full list of references, in our next issue)

The Family Allowances and National Insurance Bill has received Royal Assent and a number of changes in the Act have come into operation. For instance, children incapable of regular employment because of long-term disability can now be counted for family allowances and national insurance purposes up to the age of 16, whether or not they have been able to go to school. Previously this extension only applied to children who had never been able to go to school or who had had to leave before school-leaving age. Certain additional risks are now covered under the Industrial Injuries Scheme. An accident, occurring in the course of employment, which results from another person's misconduct, "skylarking" or negligence, or is caused by an animal, or results from the injured person being struck by an object or by lightning can be regarded as an industrial accident, provided the injured person did not contribute to the happening of the accident by any conduct or action unconnected with his employment. The Special Hardship Allowance under the Industrial Injuries Scheme is to compensate people who as a result of industrial injury or disease are unable to follow their regular occupations or employment of a similar standard. The Act extends "regular occupation" for this allowance to include employment to which people in an occupation normally advance and of which a claimant, had he not been injured, would have had at least the normal prospect. A man will now be treated as incapable of following his regular occupation so long as he is deprived of these prospects because of the effects of an industrial accident or disease.

ACUTE GASTRO-INTESTINAL ILLNESS IN GENERAL PRACTICE

BY

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Although about 5% of patients who consult family doctors in this country have symptoms of acute gastro-intestinal infections (Logan and Cushion, 1958), little information is available on the clinical and epidemiological features of these illnesses, and there are no reports of therapeutic trials in their management outside hospital. We present here a report of an investigation carried out in a group general practice, the patients of which are mainly those living on a London County Council housing estate in the north-west corner of Kent; the age-and-sex structure of the population is shown in Table I. The major part of the study was carried out during the two years 1957 and 1958, but certain aspects of it continued until the end of August, 1959. Five general practitioners took part during the first year

TABLE I.—Age and Sex of Population Studied

Age (Years)	1957			1958		
	Male	Female	Total	Male	Female	Total
0–4	380	379	759	314	326	640
5–14	1,026	1,051	2,077	756	710	1,466
15–39	1,602	1,599	3,201	1,116	1,290	2,406
40+	1,028	976	2,004	821	791	1,612
All ages	4,036	4,005	8,041	3,007	3,117	6,124

and four thereafter. The objects of the study were to investigate the clinical features, bacteriology, and epidemiology of acute gastro-intestinal disease in the home and to evaluate neomycin and phthalylsulphathiazole in treatment.

Clinical Methods

Main Survey

Every patient seen either in the home or in the consulting-room who was thought to be suffering from an acute infection of the gastro-intestinal tract or from food-poisoning was included unless, when first seen, symptoms had been present for more than seven days or antibiotics were being taken at the time of onset of the symptoms. Information about each case was recorded in a standard manner on specially printed cards, the main symptoms, the symptom that appeared first, and the symptom that was most troublesome being noted. The consumption of certain foods (cream cakes, sausages, canned meat, and reheated meat) during the 48 hours before onset, regular eating in a canteen at work or at school, contact with a similar case during the week

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