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Tissue Antibody and Complement Levels in Runt Disease. (27569)

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Runt disease, first described by Billingham and Brent(1), is induced by injection of immunologically-competent lymphoid cells from a homologous donor into an immunologically-tolerant host. The syndrome generally is regarded as the result of immunological reactions of donor cells that have proliferated in the lymphoid tissues of the host. It was considered likely that the mature and immunologically competent grafted donor cells would produce antibodies against host tissues. Studies were undertaken, therefore, to determine whether tissue antibody levels were abnormally elevated in runt disease, and additionally to test the complement (C′) levels in the affected animals.

Materials and methods. Runt disease. Newborn albino rats were inoculated with about 10^6 lymphoid cells obtained from the spleen of an adult male Canadian hooded rat; the inoculum was suspended in Tissue Culture Medium #199(2), omitting the usually added horse serum. All animals of a given litter did not experience the disease with the same degree of severity. Nevertheless, one or more of the classic symptoms of the runt disease syndrome usually was evident. Most striking of these was the relatively small size of the runted animals. In addition, some developed alopecia around the eyes and nose, and the more severely affected walked with the mincing gait characteristic of the disorder. The latter condition has been attributed to a loss of skin elasticity and tonicity which restricts normal movement of the limbs(3). Two periods of crisis were observed, the first at about the tenth postinoculation day, the second at about day 20. Although some deaths occurred with each crisis, the majority of animals surviving the second crisis lived for at least 2 weeks, and some appeared to recover completely, eventually attaining the weight of control litter mates. Prior to exsanguination (25-35 days *post partum*), inoculated animals of a given litter were segregated according to size. Those significantly smaller than control litter mates were designated "small runts." Those that approached or equalled the size of the controls were categorized "hemi-runts." Sera from a given group were pooled and stored at –35°C until used in tests.

Serological procedures. Complement fixation tests(4) employing antigens derived from tissues and organs of various animal species were used to assay tissue antibody levels in the serum pools. C′ was assayed by a precise method developed in this laboratory(5).

Results. Representative experiments are summarized in Table I. Neither liver extract antigens of albino rat, rabbit, or chicken origin, nor albino rat spleen antigen revealed a significant increase of complement-fixing tissue antibody levels in the runted animals. In addition, tests with calf thymus nucleoprotein antigen were uniformly nonreactive. Tests with liver antigen from runted animals likewise showed no increase of tissue antibody.

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TABLE I. Tissue Antibody and Complement Levels in Runt Disease of the Rat.

Serum pool No. and category	Age (days)	Complement fixation reactions* with given tissue antigens† tested with 1:3 serum: CTNP	Albino rat LSE	Rabbit LSE	Chicken LSE	Small runt LSE	Hemi-runt LSE	Normal control LSE	Albino rat spleen	Complement titers (C'_{H50}/ml)
30661–Small runt	27	—	1+	1+	1+				2+	4
Hemi-runt		—	2+	1+	1+				2+	6
Control		—	1+	±	±				1+	16
42961–Small runt	33					3+	3+	3+		6
Hemi-runt						—	±	±		20
Control						1+	1+	1+		32

		Complement fixation reactions with newborn albino rat skin antigen tested with sera diluted 1: 3	9	27	
112961–Small runt	34	3+	2+	1+	10
Hemi-runt		±	—	—	42
Control		—	—	—	44

* Degree of reaction designated 3+, 2+, or 1+; negative sign (—) indicates nonreactive.
† Identification of antigens: CTNP = Calf thymus nucleoprotein. LSE = Saline extract of liver from indicated animal species. Spleen = Saline homogenate of spleen. Skin = Saline homogenate of skin.

Intradermal inoculations with normal rat liver antigen failed to elicit detectable immediate or delayed reactions. Sera from one group of animals with acute runt disease (small runt, series 70661), on the other hand, reacted with antigen prepared from albino rat skin. Production of these anti-skin antibodies in acute runt disease may be associated with the pathological changes of the skin noted by other investigators(3).

The level of circulating C′ was markedly decreased in animals suffering from runt disease (Table I). Moreover, the degree of C′ reduction appeared to be directly related to the severity of the disorder. Particularly significant was the 4- to 5-fold decrease of C′ titer in the small runt groups. Further studies will be required to determine which of the C′ components are responsible for the decline of C′ activity. It is suggested that one or more factors may be responsible for the reduction of circulating C′: 1) C′ may be produced at normal levels but effectively removed from the circulatory system by *in vivo* fixation at the sites of antigen-antibody reactions in the host; 2) Runt disease may interfere with mechanisms essential for C′ production; 3) An anticomplementary substance may be present in abnormally high concentration. The latter may be related to serum protein abnormalities associated with runt disease previously reported(6).

Discussion. Although the preponderance of evidence supports the belief that runt disease is an immunological disorder, serologic findings differ from those observed in some of the presumed autoimmune diseases of man. For example, studies(4,7) on sera from patients with systemic lupus erythematosus have revealed significantly elevated complement-fixing tissue antibody levels, with a concomitant reduction of circulating C′. Sera from individuals with rheumatoid arthritis, on the other hand, showed no increase of tissue antibody levels, and C′ titers often exceeded normal values. These varied experiences in human and animal disease states point to the complexity of immunological disorders and suggest that several mechanisms may be involved in producing the observed syndromes.

Summary. Complement fixation tests with liver and spleen antigens derived from various animal species showed no increase of circulating tissue antibody levels in rats suffering from runt disease. Complement levels, on the other hand, were markedly decreased and degree of reduction appeared to be related to severity of the disorder.

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Influence of Dietary Stable Strontium and Calcium on the Turnover of Bone-Fixed Sr^{85} in Man.* (27570)

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Use of orally administered stable Sr and Ca to alter the distribution and retention of administered doses of radiostrontium has been studied by several investigators(1-5). These studies have indicated that retention of radiostrontium (Sr*) soon after administration is generally decreased by stable Sr or Ca given immediately prior to or immediately following Sr* administration. In this situation, the stable Sr or Ca atoms compete with the radioactive atoms being taken up by the slowly exchanging bone from the calcium pool available in blood, the soft tissues and exchangeable bone (the carrier effect).

In the almost steady-state condition which follows the transient response, the metabolic pattern changes considerably. Over 99% of the Sr* atoms which remain in the body are firmly fixed in the crystal lattice of the skeletal tissue(6). This bound Sr is henceforth subject only to radiological decay and to a slow biological turnover, resulting either from extremely slow exchange by the so-called "non-exchangeable" areas of bone, or from the bone-remodeling process (resorption).

It is thus of interest to study the effects of daily ingestion of Sr for a long period on the long-term turnover of the "fixed" Sr*, as the results cannot be predicted from the metabolic behavior of Sr* observed at early times. Such a study of the biological loss of Sr in man requires that highly sensitive measurements of the internally-deposited Sr* be made over a relatively long period, since the changes in turnover rate may be very subtle. It is difficult, if not impossible, to conduct such a study utilizing the conventional technic of radiochemical analysis. However, whole-body counting is very well suited to the purpose, as an independent, rapid and sensitive measurement of the internally-deposited gamma-emitting isotopes can easily be made, and the subjects followed for long periods.

Method. Four female patients (37 to 73 years of age) received 10 μc $Sr^{85}Cl_2$ intravenously. The patients were ambulatory and in good condition throughout the study. Diagnosis and degree of skeletal involvement in each patient are shown in Table I.

Retention of Sr^{85} was measured by *in vivo* counting with a whole-body gamma scintillation counter. The patients were positioned at a fixed geometry under an 8" × 4" NaI (T1) crystal detector as has been previously described(7). The radioactivity of the patients was determined by counting gamma-rays 4 hours after administration of the isotope and then at weekly intervals.

From 130 to 240 days following administration of Sr*, 2 patients received 1100 mg of Ca daily as gluconate, and 2 received 570 mg of Sr daily as lactate. These supplements were taken orally with meals.

Results. The biological loss of Sr^{85} in pa-

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