except at doses above 400 kr is important, since it shows that loss of micronuclei is not a major reason for reduced vigor and death after autogamy. At 200 kr about 90% of the exautogamous clones die within a few divisions, and even at 10 kr about 80% are abnormal. The animals that lose micronuclei at these doses could account for only a small fraction of these effects. The known similarity in action between x-rays and nitrogen mustard makes it appear likely that similar results would be obtained with the latter.

These data also give evidence on the process of division delay in *Paramecium*. At the lower doses used, the division of the cytoplasm and the micronuclei must have been delayed to almost the same extent. Otherwise many more animals with abnormal numbers of micronuclei would have been found. At doses of a few kiloroentgens, it is known that there is practically no delay in cytoplasmic division (*4*). The possibility remained that micronuclear mitosis might show a major inhibition comparable to that shown by the grasshopper neuroblast (*5*). If so, a large fraction of the animals should have abnormal numbers of micronuclei. Our present data suggest that this is not so, as do also Kimball's (*1*) qualitative observations on the presence of macronuclear anlagen in the progeny of paramecia given doses of a few kiloroentgens.

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A Skeletal Difference Between Sublines of the C3H Strain of Mice

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The C3H strain of mice has been used in numerous studies of genetics, cancer, and physiology for the past 25 years. It is one of the old famous strains, ranking with DBA (= dba) and C57BL (= C57blk). The strain was started in 1920 by a mating of a male from the Little strain of dilute browns (= DBA) with a female from the albino mice of H. J. Bagg (*1*). Three main sublines of C3H mice have been established. One of these is Strong's own strain at Yale University; one is Andervont's strain at the National Institutes of Health, which was separated from Strong's strain about 1930; and one is Bittner's strain at the University of Minnesota, separated from Strong's strain about 1933.

Recently it has been discovered that these sublines of C3H mice are not all alike, at least with respect to skeletal type. The purpose of this paper is to give the evidence for this claim and to call this observation to the attention of those who are using C3H mice on the supposition that the various sublines are interchangeable, or nearly so.

The number of thoracic and lumbar vertebrae may be represented by a notation such as 13/5 or 13/6. The last lumbar vertebra is sometimes modified so that on one side it resembles a sacral vertebra. These intergrades are called "asymmetrical."

The skeletal type of C3H mice was first observed in a sample of 200 descended from mice obtained from Strong in 1937. About 96 per cent of these were 13/5, 3 per cent were asymmetrical, and 1 per cent were 13/6 (*2*). A second sample of 767 was produced from mice obtained from Bittner at the Jackson Laboratory in 1939. This sample gave a distribution of skeletal types almost identical with the first. The two samples were combined into a single sample of 967 (*3*). In 1948–52, three samples of C3H mice were obtained, all of which trace to the Andervont subline of C3H (more recently called the Heston subline, C3H/He). These three samples were similar, having about 96 per cent 13/6, but all were markedly different from the previous samples. In 1950–52, fresh samples of 144 and 75 from Bittner's C3H and C3H fostered on C57BL were obtained. These were not exactly like any of the preceding sublines, but did rather closely resemble the C3H mice obtained from Bittner and from Strong. In May 1952, a preserved litter of 7 C3H mice was obtained from Wilson. These were descendants of C3H mice received from Strong in 1947. All 7 were 13/5. The data are summarized in Table 1. For comparison, data are included on the CBA strain, which Strong derived from the same cross that produced C3H. The sample consists of 153 CBA mice, descended from mice obtained from Carter,

TABLE 1

DISTRIBUTION OF SKELETAL TYPES IN SEVERAL SAMPLES OF C3H MICE AND IN ONE SAMPLE OF CBA MICE

Strain	Origin	Thoracic/lumbar vertebrae (%)				Number
		13/5	Asym	13/6	Other	
C3H	Strong in 1937	96	3	1	0	200
C3H	Bittner in 1939 (= Bittner's Z)	97	2	1	0.1	767
C3H/He	Law in 1948	2	2	96	0.6	177
C3H/HeJax	Jackson in 1950	0	3	97	0	30
C3H/HeRl	Russell in 1950	1	3	96	0	72
C3H/Bi	Bittner in 1950 (= Bittner's Z)	93	6	1	0	144
C3Hb/Bi	Bittner in 1950 (= Bittner's Zb)	88	1	11	0	75
C3H/Wi	Wilson in 1952	100	0	0	0	7
CBA/Ca-*se*	Carter in 1950	95*	3	2	0	153

* Includes 10 *sese* mice of type 12/6 and 40 *sese* mice of type 13/5.

50 of which are homozygous for a new mutation to short ear (*se*). Ten of these *sese* are 12/6 (the reduction in rib number being associated with *se* [*4*]), but otherwise the CBA mice are like the C3H sublines of Strong and Bittner.

No explanation of the observed difference is known. On the assumption that the difference is genetic, the following are possibilities. (1) The original C3H mice may have been heterozygous for one or more pairs of genes affecting skeletal type. By inbreeding, different genetic combinations may have become fixed in different sublines. The heterozygosity would have had to continue in the line at least until the Andervont subline was established in 1930. (2) One or more mutations of skeletal genes may have occurred following the separation of the Andervont subline, leading thereby to the establishment of different types in the existing sublines. (3) One of the sublines may have been genetically contaminated by an accidental and unrecorded mating outside the line. (4) Some other strain may erroneously have been labeled as C3H.

Pursuit of the origin of the difference appears fruitless. The important point is that, pending further information, at least two distinct types of C3H mice must be recognized. They may be designated as C3H/St or C3H/Bi for Strong's and Bittner's sublines and as C3H/He for the Andervont (Heston) subline.

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Extraction of the Hyperglycemic Factor (HGF) of the Pancreas with Liquid Ammonia[1]

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The intravenous injection of most commercial insulin preparations is followed by a short period of hyperglycemia which reaches a maximum in 10–15 min, then gradually gives way to the typical insulin hypoglycemia. This phenomenon was known to early investigators who, working with pancreatic extracts but being mainly concerned with the purification of insulin, attributed it to an undesirable "contaminant," difficult to eliminate. Later investigators became interested in this "contaminant" per se, separated it from insulin, and called it "glucagon" or the "hyperglycemic-glycogenolytic factor (HGF)." Its properties have been extensively reviewed (*1–4*) and have led some investigators to suggest that HGF is a second pancreatic hormone, possibly secreted by the alpha cells of the islets of Langerhans (*5–8*). Other investigators (*9, 10*) have cautioned against the premature acceptance of this conclusion and have pointed out that, although HGF might actually be a hormone, direct proof of its secretion *in vivo* is not yet available. Indeed, the possibility exists that HGF might be a cleavage product of the insulin molecule, since most HGF preparations have been obtained by the rather drastic procedure of destroying insulin with alkali at 39° C (*11, 12*). In a few instances insulin has been inactivated by reducing its —S—S— linkages with cysteine, but the complete inactivation requires a ratio of cysteine to insulin of 40 : 1 by weight (*13*), and the physiological properties of such preparations are hard to evaluate in view of the hyperglycemic effect of cysteine itself (*14*). A method for the preparation of HGF that would avoid these difficulties would therefore be desirable.

Liquid ammonia dissolves many proteins, including insulin, with minimum denaturation and little or no loss of physiological activity (*13, 15, 16*). Furthermore, insulin dissolved in liquid ammonia is inactivated by a ratio of cysteine to insulin of only 1 : 1 by weight (*17*). Since insulin and HGF have very similar chemical properties, an attempt was made to extract them both with liquid ammonia. Insulin could then be inactivated by an amount of cysteine calculated on the basis of the estimated insulin content of the pancreas used.

Granulated lyophilized pork pancreas was ground in a Waring blendor; 200 g of the powder was placed in a transparent 1-liter Dewar flask graduated in 100-ml divisions and extracted with five successive 200-ml portions of liquid ammonia delivered directly from the original commercial cylinder. Each portion was left in contact with the pancreas for about 30 min; during this period the boiling of the ammonia kept the suspenison under continuous mild agitation. A second 1-liter Dewar flask, containing 50 mg of cysteine hydrochloride, was connected with the first one by means of a glass tube attached to a glass wool filter. At the end of each 30-min period, the clear pink solution was pumped from the first to the second flask by means of a rubber bulb. The cysteine hydrochloride was placed in the receiving flask before the extraction was started, to minimize handling and because the addition of the crystals to the liquid ammonia causes a violent boiling over of the solution. The cysteine readily dissolved in liquid ammonia and was found sufficient to inactivate completely the insulin extracted from the 200 g of pancreas powder. Care was taken to avoid leakage of water into the flasks; water, which constantly condensed and froze on the rubber and glass connections, would have caused the formation of ammonium hydroxide, and this might have dena-

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