ilar products), but in the pulp of macerated tissues used for cultivation he introduces large amounts of cell constituents of both high and low molecular weight. They may be removed by repeated washings either before or after they are placed in the culture tubes, and thus the difference between his results with dextrose and sucrose may be explained. The active growth of his cultures of tissue cells indicates the presence of the growth-promoting substance "embryonin" in his media, this substance being present in the macerated cells. A comparison with controls consisting of cultures made from washed tissue pulp would have been desirable.

Summary. 1. The difference between growth-promoting substances and accessory growth substances and between their different modes of action is pointed out. 2. From dialysates from embryo juice, preparations may be obtained which completely restore the ability of the dialysed culture media to induce normal growth of the tissue cultures. They may be prepared free from organic phosphates and glutamine. 3. A synthetic mixture is described which has the same properties of restoring the dialysed media. The most important of its components seem to be glutamine and fructose-diphosphate.

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Hereditary Dwarfism in the Descendants of Mice Receiving Methylcholanthrene—Parallel Induction.

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The following biological variants have been obtained in the descendants of mice whose ancestry have been injected with methylcholanthrene for many generations. (1) Several germinal mutations changing susceptibility to tumors induced at the site of the injection of the carcinogen. (2) Germinal mutations altering the incidence of specific types of tumors, following the injection of the methylcholanthrene and subsequently under spontaneous conditions in their untreated descendants—such as bronchiogenic carcinoma, leiomyo-sarcoma of the uterus, and gastric lesions of several histological types. (3) Many embryological disturbances such as dextrocardia, dextroversion, situs inversus, anovariae, absence of the cervix and vagina, absence of one kidney together with the absence of the horn of the uterus on the same side, craniorachichisis, several eye lesions, and possibly (?) twins. (Twenty-two pairs of twins have been obtained in mice which have been injected with methylcholanthrene. In view of the reported rarity of this condition in mice (only three cases could be found in the literature) it would seem that twinning in these methylcholanthrene-treated mice is highly significant. However, in more recent work especially by W. F. Hollander,[1] it has been found that twinning or placental fusions in mice are of very frequent occurrence. It is doubtful, therefore, whether the injected methylcholanthrene had much influence on the development of this biological variant.) The above biological variants are considered to be induced by methylcholanthrene when they have fulfilled one of the following criteria: either (a) they have occurred only among the injected mice or their immediate descendants and never among a comparable group of controls, or (b) they have occurred among the methylcholanthrene-treated mice at an increased frequency over the controls. (4) Several physiological and morphological charac-

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[1] Hollander, W. F., unpublished data.

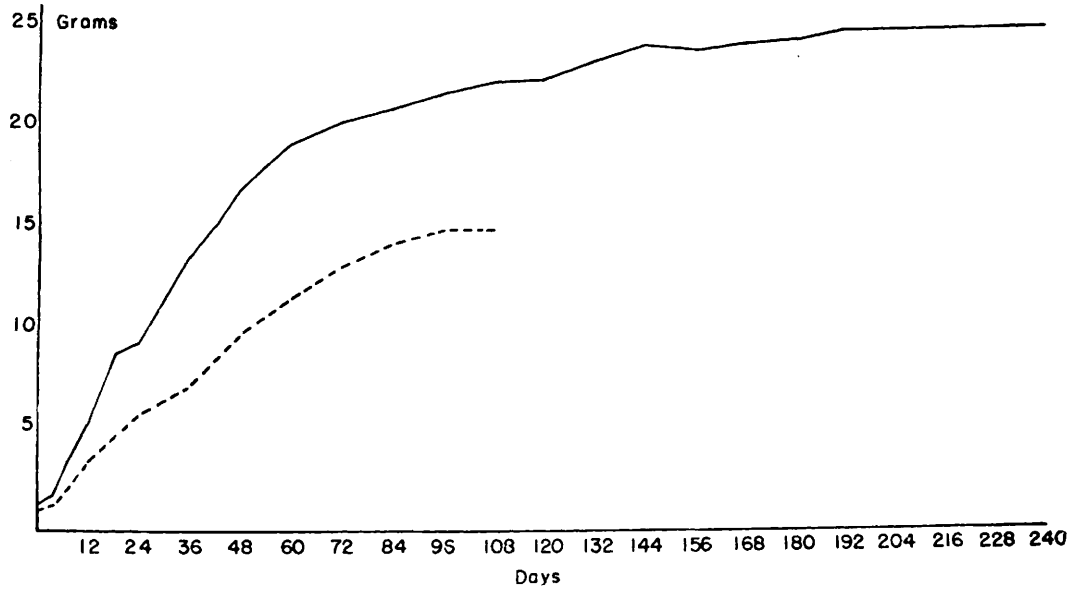

CHART 1.

This chart presents data on the growth rate of (a) normal male mice of the pBr subline of the NHO stock (solid line) and (b) hereditary dwarf mice of the same stock (short dash line). Time in days is given on the base line, body weight in grams is given on the vertical line.

teristics such as precocious opening of the vagina, delayed opening of the vagina even up to one year of age, and complete imperforate vagina, increased fecundity, precocious sexual maturity, increased fertility, excessively large first litters, rapid growth rate with the production of giant mice weighing up to 86 g of weight, and precocious and complete sterility. (5) Numerous cases of somatic mosaics especially involving hair pigmentation, and (6) the production of many germinal mutations involving hair color, eye color and distributional patterns of pigmentation.[2-8]

Recently Carr[9] has reported 7 biological variants produced in mice by the subcutaneous injection of 1:2:5:6-dibenzanthracene. He reports 1. Hydrocephalus, 2. Absence of left horn of the uterus, 3. Brain hernia, 4. Brown, 5. Pink eye, 6. Recessive spotting, and 7. Chinchilla. The last 4 of these variants were proven to be genetic recessives.

It is a well recognized fact that the carcinogens are inhibitory in action.[10] Thus when one injects a mouse with one of the carcinogens the normal growth rate is inhibited. In the pBr subline of the NHO descent whose ancestry has been injected with methylcholanthrene for many generations and the mice selected toward induced cancer resistance, the mice very seldom weigh more than 25 g when fully grown. The normal growth rate for mice of this subline is given on the solid line of Chart 1. On the 31st of March, 1947, it was noted that a pair of these pBr mice had produced a litter of young in which there were 4 normal and one dwarf mouse. These mice and their close relatives have produced, to date, a total of 121 mice in 21 litters in which, at least, one dwarf has appeared. Of these 121 mice, 92 were normal and 29 dwarfs. On a 3:1 basis one would expect 90.6 normals to 30.2 dwarfs. Thus it is obvious that dwarfism is a simple mendelian recessive.

2 Strong, L. C., *Arch. Path.*, 1945, **39**, 232.

3 Strong, L. C., *J. Nat. Cancer Inst.*, 1945, **5**, 339.

4 Strong, L. C., *Nat. Acad. Sciences*, 1945, **31**, 290.

5 Strong, L. C., *Yale J. Biol. and Med.*, 1946, **18**, 145.

6 Strong, L. C., *Yale J. Biol. and Med.*, 1946, **18**, 359.

7 Strong, L. C., *Science*, 1946, **103**, 554.

8 Strong, L. C., *American Naturalist*, 1947, **81**, 50.

9 Carr, J. C., *Brit. J. Cancer*, 1947, **1**, 152.

10 Boyland, E., Edgar Allen Memorial Lecture, New Haven, Conn., Oct. 8, 1947.

The growth rate for dwarf mice up to 108 days of age is given on the short dash line of Chart 1.

The dwarfs are smaller in size than the controls at birth and appear to be restless. They deviate strikingly from the controls at 2 to 3 days of age and, in competition with their normal siblings, they have invariably died before the weaning age of 30 days. A few days before death they lose weight progressively. However, when their normal siblings have beeen removed from the mother, the dwarfs will live for a longer time. So far only 5 have reached 100 days of age. Of these one pair has reached sexual maturity and has produced 16 young in 2 litters—all of which were dwarfs. One of these young was also a waltzer but unfortunately died before reaching sexual maturity, so that the biological nature of this waltzing could not be determined.

It is perhaps more than a coincidence that the mutations reported by Carr are the same as have been obtained in my laboratory. All 4 of his mutants have occurred several times in the descendants of mice which have been injected with methylcholanthrene. In addition, however, I have obtained many other germinal mutations, notably three types of dominant spotting, etc. One may entertain the possibility that perhaps there may be a specificity of action of the inducing system upon the genetic mechanism, an idea that has been mentioned previously. It must be borne in mind, however, that the first mutations that have been induced with methylcholanthrene and with 1:2:5:6-dibenzanthracene have been at loci which have given rise to spontaneous mutations in the past—thus indicating perhaps a greater mutability than other loci in the germ plasm of mice. The greatest difference between Carr's data and my own is, apparently, in the greater frequency of dominant mutations in my series. For example, with methylcholanthrene the recessive gene b has mutated to dominant B eight times, whereas the gene B has only mutated to b once. It is also clear that both non-genetic and genetic biological variants are being produced in separate laboratories. In mice, many biological variants occur for which no genetic evidence of inheritance can be obtained. It is consequently better to use the term biological variant until evidence of inheritance can be established. When this evidence is forthcoming the variant thus becomes a mutant. Among the most interesting non-genetic variants are the somatic mosaics being induced with methylcholanthrene. The fact that both methylcholanthrene and 1:2:5:6-dibenzanthracene are powerful carcinogens and that consequently the experimental animals are giving rise to a multiplicity of cancers should not be lost sight of.

Parallel induction maintains that an induction system may influence both the soma and the germ plasm—perhaps conditioned by some biological bond between the gene and some characteristic of the soma conditioned by gene action. This appears to be the case in the induction of gastric lesions under the influence of methylcholanthrene. This gastric lesion became hereditarily established by a mutation on the brown tagged chromosome.[5] The production of hereditary dwarfism in mice seems to be a second case of parallel induction. That is, that the induction system of methylcholanthrene or one of its metabolites has inhibited the normal growth rate of mice for several generations. When a germinal mutation took place it took place in the direction of the inductive influence. However, this has not always been the case. For example, the giant mice referred to previously apparently originated by some biological change (perhaps germinal) which was counter to the trend of biological induction.

The embryological disturbances may be brought about, perhaps, by an inhibitory action of the carcinogen at a critical period of the development of the embryo. For example, the absence of the cervix could be explained by the fact that perhaps the distal end of the Müllerian duct did not develop normally. The carcinogen is also inducing mutations and at the same time bringing about changes in the soma leading toward neoplasia. This multiplicity of biological effects by the injection of a pure compound may eventually be better understood when the metabolism of the carcinogen is more fully understood.

Summary. Among the germinal mutations

induced in mice following the subcutaneous injection of methylcholanthrene for many generations is hereditary dwarfism. This condition is inherited as a recessive. The relation of this mutation to the general problem of parallel induction is briefly indicated. Thus it is becoming more obvious that both non-genetic and genetic biological variants are being produced in mice by methylcholanthrene. The mechanism involved may be different for the different variants obtained but perhaps there may be a common denominator in them all.

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Effect of Muscular Fatigue on Histamine-Provoked Ulcer with Observations on Gastric Secretion.

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Complete physical rest is frequently mentioned by internists as a valuable adjunct in the medical management of peptic ulceration. This precept has been derived primarily from clinical observation as there are few if any experimental studies in recent medical literature which deal with the effects of muscular fatigue on alterations in gastrointestinal tract function particularly as they pertain to the ulcer diathesis.

It is the purpose of this report to indicate our observations on the effect of moderately severe muscular fatigue on the incidence of the histamine-provoked ulcer and upon gastric secretion in dogs.

Method of Study. Experiment 1. Fifteen healthy, dewormed dogs weighing from 26 to 54 lb, of young to medium age, were run from 3 to 4 hours consecutively each day in the morning on 5 to 6 successive days on an electrically driven endless belt treadmill. The dogs were allowed a 10-minute rest period each hour and water *ad lib.* The treadmill speed was calculated at 1.5 to 2.0 miles per hour which amounts to a brisk walking pace for the average sized dog. The slope of the treadmill was varied between an incline of 5 to 20° from horizontal depending on the dogs' physical condition. Just prior to being placed on the treadmill each day, the dogs were given an injection intramuscularly of 30 mg of histamine base-in-beeswax mixture prepared after the method of Code and Varco.[1] The dogs were fed a diet of horsemeat, milk, dog biscuit each day after finishing their period on the treadmill. Rectal temperatures were taken before the start of the exercise and after each hour of exercise as a gross indication of the degree of muscular effort.

As a control series, 13 animals of comparable size, age, and health were fed and injected daily with histamine as above, but were not run on the treadmill. In addition 2 dogs were run on the treadmill as above, but were not given histamine. In these 2 animals the effect of the muscular exercise on hemoglobin, hematocrit, leukocytes differential count, and blood sugar was studied. All animals were sacrificed with examination of their gastro-intestinal tracts at the end of their test period of 5 to 6 days on the treadmill and/or 5 to 6 consecutive daily intramuscular histamine-in-wax injections.

Experiment II. Studies on gastric pouch secretion were made using 2 Heidenhain-type pouches made over one year prior to the onset of these experiments. Both animals used were

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1 Code, C. F., and Varco, R. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 475.