the 5 pairs were involved. In 13 of the 18 cells an abnormally large ("marker") and/or small chromosome was present. Some of the large marker chromosomes were of subterminal morphology.

Discussion. The data above indicate that the subterminal chromosomes are more vulnerable to alterations than are the median or terminal chromosomes. The reason(s) for this differential susceptibility is now known, but it may reflect some property of the subterminal structure or it may be that similar alterations in other chromosomes are less compatible with cell survival. Possibly the difference can be explained by the fact that a subterminal chromosome can become terminal by loss of the upper or lower arms or median by loss of a portion of the lower arms.

Although the distribution of chromosome number of cells in the various sublines corresponded to that of the respective cell line, no distinction between the 2 cell lines could be based on the karyotype changes observed. Morphologic differences in cells and in colonies of the 2 cell lines and sublines have been observed, however. Whether or not the chromosome distribution differences are due to differences in the carcinogen used is a matter for further studies. Studies of cells directly from the cancers are now in progress.

Summary. Permanent lines of cells cultured from a 3-methylcholanthrene and a 7,12-dimethylbenzanthracene induced rat mammary cancer have been obtained. Although the most common chromosome number of cells in both lines was the diploid number, the 3-MC line had many hypodiploid cells and the DMBA line had many tetraploid and hypotetraploid cells. Deviations from the normal rat karyotype were the rule even in diploid cells. The most common alteration was absence of subterminal chromosomes.

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Determination of Serum Amylase by Measurement of Maltose and Other Products from Starch Substrate. (29722)

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Action of serum, urine, pancreatic or salivary α-amylase on starch produces scission of starch molecules into progressively smaller sugar units. This process occurs through a sequence of steps, producing reducing dextrins which then are split into smaller molecules of oligosaccharides with reducing properties, among them being maltotetraose, maltotriose, maltose and possibly maltohexose (1-5). Henry and Chiamori published the results of a study of the saccharogenic method for determination of serum and urine amylase(1) and proposed a modification of Somogyi's method(6) in which conditions of incubation of starch with urine or serum were improved. After 30 minutes' incubation, the reducing capacity is determined using the Folin-Wu(7,8) or Nelson-Somogyi(9) method. Amylase activity in this method is the amount of reducing substances as mg glucose produced in 30 minutes at 40°C per 100 ml serum, which is equal to the number of amylase units. The enzyme activity, therefore, is measured as the difference in reducing substances present before and after incubation with substrate. It would be preferable if one could measure only one or more substances formed in the reaction and not pres-

ent in appreciable quantities prior to incubation. Fearon introduced a color reaction for detection of lactose(10) in which a red color is produced by heating the aqueous solution of this sugar with ethylamine. He pointed out that the main interference in this reaction is maltose which produces almost the same color. Lactose is not produced during enzymatic hydrolysis of starch and it has been found that, by adapting the reaction to quantitative determination of maltose, it is possible to determine amylase activity by measuring the rate of formation of products reacting as maltose.

Method. 1. Phosphate buffer, 0.1 M, pH 7.0. Dissolve 4.55 g KH_2PO_4 and 9.35 g Na_2HPO_4 in water and dilute to 11. 2. Starch substrate. Dissolve 75 mg soluble starch according to Zulkowsky (E. Merck, Germany) in 3.5 ml phosphate buffer just before use. 3. Arsenious acid-potassium iodide solution. Dissolve 1 g As_2O_3 in 100 ml hot water, then cool to room temperature and add 5 g potassium iodide. Adjust the volume again to 100 ml. 4. Ethylamine hydrochloride. Dissolve 10 g in 100 ml water. 5. Sodium hydroxide. 20% aqueous solution. 6. Maltose standard, stock solution. 1% in saturated aqueous solution of benzoic acid. Stable for at least 3 months. 7. Maltose standard, working standard. One ml stock standard plus 13.0 ml substrate. 3.5 ml = 2.5 mg maltose. Prepare freshly. *Procedure.* Add 0.2 ml serum or plasma from a TC pipette to 3.5 ml substrate in a test tube labeled "test" and incubate in a water bath at 37°C. After exactly 30 minutes, remove from the bath and immediately add 1.0 ml arsenious acid-potassium iodide solution, 0.5 ml ethylamine and place the tube immediately in a boiling water bath for 15 minutes. Prepare a "standard" and a "blank" as follows: To 3.5 ml working standard solution add 0.2 ml serum or plasma, 1.0 ml arsenious acid-potassium iodide solution, and 0.5 ml ethylamine reagent for the "standard." The "blank" is prepared in the same way, substituting 3.5 ml substrate for the 3.5 ml "working standard" solution. The tubes are placed in boiling water without delay. After 15 minutes, the tubes are removed from the water bath one at a time and immediately 0.5 ml 20% sodium hydroxide is added to each tube while still hot. Allow tubes to cool to room temperature and read absorbance of the "test" (A_x), "standard" (A_s) and "blank" (A_b) against water at 535 mμ. The amylase activity expressed as μmoles of maltose equivalent produced per minute per ml sample at 37°C is:

$$\text{Amylase units} = \frac{A_x - A_b}{A_s - A_b} \times 2.5 \times 5 \times \frac{1}{30} \times \frac{1000}{360}$$

$$= \frac{A_x - A_b}{A_s - A_b} \times 1.16.$$

Results. Fig. 1 shows the absorption curve of the colored product formed in the reaction. The absorption peak is at 535 mμ. The sensitivity of the color reaction under the conditions of the method presented is about 0.200 absorbance unit per mg maltose for a 1 cm light path. No deviation from Beer's Law was observed with a Beckman model B spectrophotometer at 535 mμ or a Klett photometer using a no. 54 filter. At absorbances greater than 0.5 the color was found to be stable for at least 2 hours, but the color was less stable at lower absorbances, necessitating reading within 10-15 minutes.

Increments of 2.5 and 5.0 mg maltose were added to 3 different sera prior to incubation. Mean recoveries were 99 and 100%, respectively.

Fig. 2 shows that there is a linear relation between the absorbance of the color produced and time of incubation of a serum with the substrate for a period of at least 1 hour. The first point of the line at zero time corresponds to the absorbance of the "blank" (A_b) in the analytical procedure.

A serum sample of high amylase level was used in the experiment illustrated in Fig. 3 where increasing volumes of serum (diluted to 0.2 ml with saline) were added to different substrate tubes, incubated in a water bath at 37°C for 30 minutes, and the color developed as described in the method. A linear relationship was obtained between absorbance and volume of serum used, the slope of the line being proportional to the amylase activity of the given serum. The first point of the line corresponds to the absorbance of a reagent blank, *i.e.,* where 0.2 ml of saline was added instead of a diluted serum.

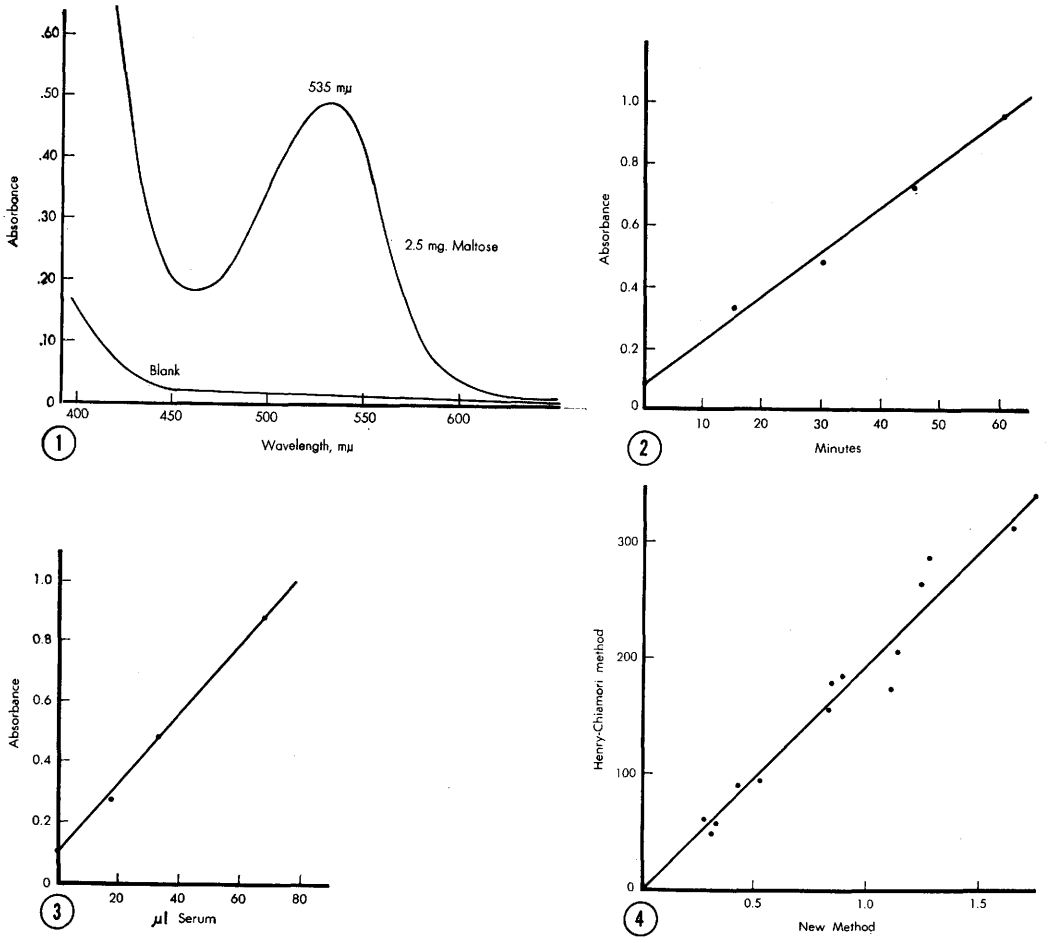

FIG. 1. Spectrum of maltose-ethylamine reaction product.
FIG. 2. Influence of incubation time.
FIG. 3. Influence of amylase concentration.
FIG. 4. Regression line relating results by the Henry-Chiamori method (Somogyi units) to the present method (new units).

Interference by other sugars was checked by replacing in the standard maltose solution the equivalent amount of glucose, xylose, fructose, ribose, lactose, sucrose and galactose. Lactose produced a color equivalent to maltose; no color was produced by the other sugars. Unfortunately, maltotriose, maltotetraose, maltohexose and other oligosaccharides related to maltose were not available for testing.

The standard deviation as determined from duplicate analyses of 13 sera was 0.056 unit giving 95% precision limits (2.16 C.V.) of ± 11%.

The present method was compared with the Henry-Chiamori modification of the Somogyi method(1). The correlation coefficient obtained for results by the 2 methods on 13 sera ranging from 47 to 314 Somogyi units was 0.975, indicating excellent agreement. The mean ratio of Somogyi units (Henry-Chiamori modification of Somogyi method) to amylase units (present method) for the 13 samples was 197, *i.e.*, 1 amylase unit is equivalent to 197 Somogyi units. The normal ranges (95% limits) by the modified Somogyi method (1) were found to be 34-118 and 46-141 units per 100 ml for males and females, respectively. Hence the normal ranges for the new method would be 0.17-0.57 unit for males and 0.22-0.68 unit for females.

The calculated slope of the regression line

(y on x) relating results by the Henry-Chiamori (y) to the present method (x) was 202 (Fig. 4). The theoretical value for this slope, assuming that only maltose is measured in both methods, is 490 (approximate glucose equivalent for maltose in the Folin-Wu method taken as 0.4 and making a correction of + 13.5% for the difference in temperatures used in the 2 methods). Since it has been definitely established(1) that products other than maltose are measured in the Henry-Chiamori method the slope would be greater than 490 if only maltose was being measured in the present method. The finding of a slope of 202, therefore, indicates that (1) substance or substances other than maltose formed in the enzymatic hydrolysis are being measured by the ethylamine reaction, (2) the ratio of the absorptivity of color produced with ethylamine to the respective reducing capacity of this substance or substances is higher than the corresponding ratio for maltose.

Summary. A modification of Somogyi's saccharogenic method for serum amylase is presented in which maltose and other products formed in the enzymatic hydrolysis of starch are determined photometrically by reaction with ethylamine. Various aspects of this method were studied and results compared with the Henry-Chiamori modification of Somogyi's method.

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Rat Virus (RV) Infections of Pregnant, Fetal and Newborn Rats.* (29723)

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The present study concerns the pathogenesis of rat virus (RV) infections in rats. It is also a part of a continuing one on viral infections of pregnant, fetal and newborn animals, using rat(1) and H-1(2) viruses to explore circumstances under which congenital malformations and vertical transmission of viruses and possibly neoplastic transformations may take place. RV has attributes which suggest usefulness as a probe in these investigations. In addition to its unusually small size of 13 mμ(3), it has an ability to cross the placental barrier in hamsters(4) as well as to induce malformations in tissues and organs which continue their development after birth. Such tissues include the teeth, where RV may produce markedly different effects, depending on the stage of ontogenesis of the molars at time of inoculation(5); second, a generalized effect on skeletal development as shown in mongoloid dwarfism(6), and third, a cerebellar hypoplasia and ataxia resulting from destruction of the granular cell layer, following inoculation at birth(7).

The related H-1 virus is in the same size range as RV(3). It is a natural, latent virus of rats with many attributes similar to those of RV as well as a marked capacity for induction of congenital malformations in hamsters(8). It is planned to compare the pathogenesis of these two closely related parasites of rats in continuing studies.

Materials and methods. Virus. Of the 2 strains of rat virus employed, the 171 strain

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