It is of interest to point out that certain human plasma fractions (III-0, IV-1 and albumin) which inhibit bacterial modification of red cells also bring forth agglutination of red blood cells in the presence of incomplete Rh antibodies(7). Whether these 2 phenomena have an underlying mechanism in common remains to be determined.

*Summary.* 1. The modification of red blood cells by boiled suspensions of *E. coli* serogroups 026, 055 and 0111 is inhibited by human and animal sera as well as by egg yolk and various fractions of rat liver. Certain human plasma fractions in concentration of 0.1% (particularly fractions IV-1, IV-7 and albumin) are likewise effective, whereas others are not. 2. This inhibition is due largely to the action of the inhibitor on the bacterial antigen rather than on the red blood cell. 3. The significance of the results is discussed.

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## XYZ Effect in Strain of Origin: EO771 Carcinoma in C57 BL/6 Mice. (19708)

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The xyz factors(1-11) are specific agents present in long-frozen or lyophilized tissue or fresh supernatant fluid from certain malignant tumors which, when injected, render the animal hosts more susceptible to the subsequent transplant of the same tumor. If the xyz factors are to affect the malignancy of spontaneous tumors in nature then it must be shown that each factor in transplanted tumors will influence the course of the same neoplasm in the inbred strain of origin. Prior experiments have shown that the EO771 factor would enhance the course of malignant disease in the C57 black strain(7,8) as well as in foreign strains of mice(5,6,11). It did not affect the course of transplanted tumor growth in C57 blacks for 3 other mammary cancers which arose in C57 black mice (755, 241-5, 241-16) nor did extracts of the other 3 tumors affect the course of EO771 in C57 blacks (7,8). This seemed significant, particularly since the EO771 xyz factor has been demonstrated in some concentration in the normal liver and kidney of the Jackson Laboratory strain of C57 blacks but not in normal spleen (10,11). Mammary cancers 755, 241-5, 241-16 had been carried in the National Cancer Institute strain of C57 blacks, and although the EO771 tumors used came from the Jackson Laboratory the experiments were carried out with the Carworth Farms strain of C57 black mice. The possibility arose that the diversity of C57 black strains used might have influenced the results. The present paper reports experiments on the effect of the EO771 xyz factor obtained from tumors inoculated in the Jackson Memorial Laboratory and tested on C57 BL/6 mice obtained from the same source.

*Materials and methods.* Fifty-two C57 BL/6 mice, 3-4 months of age, were obtained in one batch from the Jackson Memorial Laboratory in Bar Harbor, and were caged in 8 boxes of 6 or 7 mice in each.

Twenty-six mice (7 males and 19 females) were injected subcutaneously into the left

TABLE I. Rapidity of Tumor Growth and Mortality from Mammary Carcinoma EO771 in 52 C57 Black/6 (Jax) Mice, 26 of Which Had Been Pretreated with Frozen EO771 Tumor Tissue.

| Exp. # | Mice (#, sex) | Breed | Treatment: Sr[x] | Treatment: Days[a] | Treatment: Days[b] | Inoc. source | Median diam., cm* | Died | Med. day† | Mortality days |
|---|---|---|---|---|---|---|---|---|---|---|
| 5069 | 6 ♀ | BL | $BL^1$ | 49 | 32 | $BL^2$ | 1.7 | 6 | 20 | 14, 19, 19, 21, 22, 25 |
| 5013 | 7 ♂ | " | $BL^1$ | 49 | 32 | " | 1.9 | 7 | 18 | 14, 17, 18, 18, 19, 25, 41 |
| 5006 | 7 ♂ | " | — | — | — | " | 1.4 | 7 | 22 | 20, 21, 22, 22, 24, 35, 43 |
| 5082 | 6 ♀ | " | — | — | — | " | 1.4 | 6 | 26 | 21, 23, 25, 26, 29, 42 |
| 5075 | 7 ♀ | " | $BL^1$ | 49 | 34 | $BL^3$ | 1.5 | 7 | 22 | 14, 17, 20, 22, 24, 25, 28 |
| 5089 | 6 ♀ | " | $BL^1$ | 49 | 34 | " | 1.8 | 6 | 21 | 15, 20, 20, 21, 21, 21 |
| 5095 | 7 ♀ | " | — | — | — | " | 1.3 | 7 | 23 | 23, 23, 23, 23, 24, 26, 36 |
| 5102 | 6 ♀ | " | — | — | — | " | 1.4 | 6 | 22 | 19, 20, 20, 23, 26, 30 |

* Median diameter of the tumors measured in 3 dimensions at 15 days.

† Median day of death for the group.

BL—C57 BL/6 (Jax) mice; Sr[x]—breed of mouse from which EO771 tumor was taken for freezing; $BL^1$—C57 BL/6 (Jax) mouse; Days[a]—length of time tumor kept frozen at 0°F; Days[b]—interval between inj. of frozen tumor tissue and tumor transplantation; Inoc. source—source of EO771 tumor used for transplantation; $BL^2$ and $BL^3$—C57 BL/6 (Jax) mice from which EO771 tumor was taken.

groin with 0.1 cc of a 1-3 dilution of frozen tumor tissue (xyz factor) in normal saline (Groups 5069, 5013, 5075, 5089; Table I). No tumors or palpable reaction occurred at the site of the injections. The tumor tissue had been stored aseptically in 50% glycerin in normal saline for 49 days at 0°F and had been taken from a C57 BL/6 mouse inoculated with EO771 in the inbred nucleus at the Jackson Laboratory (all tumors from the Jackson Laboratory shipped through the courtesy of Dr. George Snell). The remaining 26 mice (7 males, 19 females) were controls, receiving no treatment prior to transplantation of viable tumor (Groups 5006, 5083, 5095, 5102).

The 52 mice were redivided into 2 groups of 26 mice in each so that each new group contained 13 experimental and 13 control mice. Two C57 BL/6 mice carrying EO771 tumor, received from the inbred nucleus at the Jackson Laboratory, were used for the subcutaneous transplantation of the 52 mice (Table I). The tumor tissue was prepared by mincing and grinding without sand with the addition of 3 parts of normal saline; 0.1 cc of the emulsion was injected subcutaneously into the left groin in one group and into the right groin in the other group. The transplants were successful and all 52 mice died from tumor growth with 14-42 days after inoculation. Measurements were taken of the growth in 3 dimensions twice weekly and the mortality was plotted in days after the tumor transplantation. The "volume" of the tumor was calculated for each mouse at 15 days by using the product of the diameters in 3 dimensions. (Three mice died on the 14th day).

Although all 52 mice died from tumor growth, the 26 mice pretreated with the nonviable frozen tumor tissue developed tumors which grew more rapidly and killed sooner than was the case among the 26 control mice (Fig. 1, 2). The mean volume of the primary tumor at 15 days was 3.26 ccm for the 26 controls (sum $X^2 = 428.6$, var. $m_x = 0.23$) and 6.62 ccm for the 26 mice pretreated with frozen tumor tissue (sum $X^2 = 1397.6$, var. $m_x = 0.40$). The difference of $3.4 \pm 0.8$ ccm was statistically significant ($t = 4.24$, $n = 50$, $P = 0.001$-). The mortality according to days after inoculation was significant when calculated by the method of Shear, Imagawa, Syverton, and Bittner(9). When 50% of the treated group had died only 13% of the controls were dead, and when 50% of the controls were dead 78% of the treated animals had succumbed.

*Discussion.* Since the first paper on the testing of xyz factors in the inbred strain of origin(8) Shear, Imagawa, Syverton, and Bittner have demonstrated that the xyz factor present in Z (C3H mice, Bittner) tumor 8352 is effective in influencing the course of the same neoplasm in the inbred strain of origin(9).

*Summary and conclusions.* Fifty-two C57

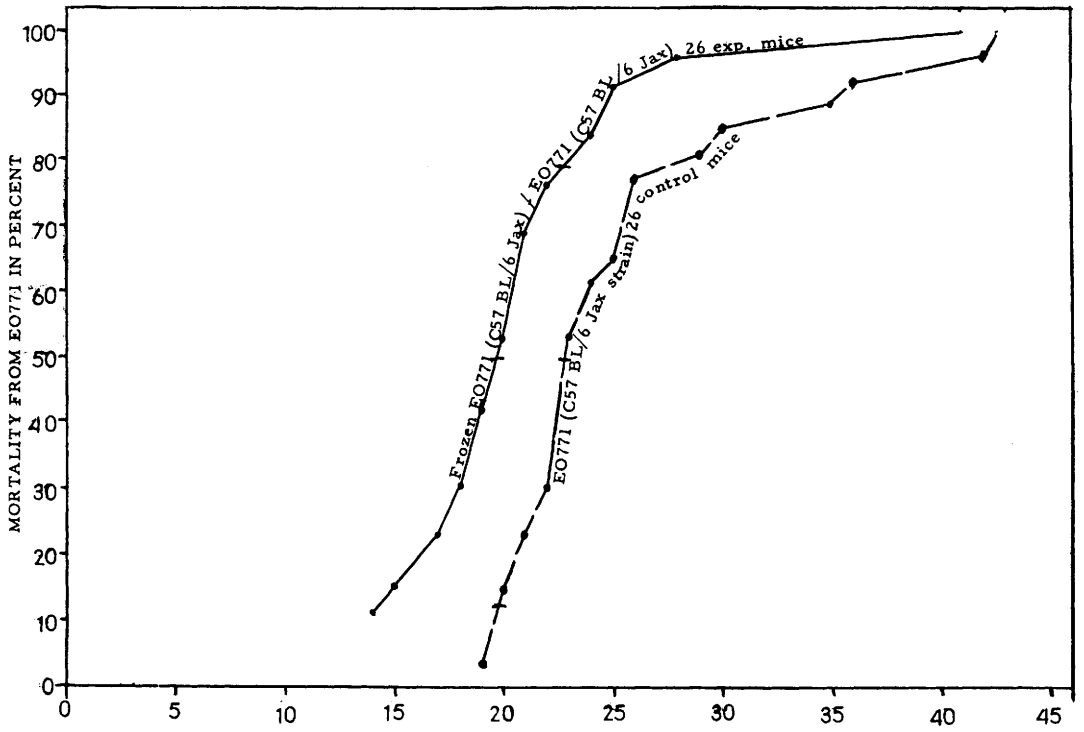


FIG. 1. Days after transplantation of EO771 tumor with C57 BL/6 (Jax) mice.

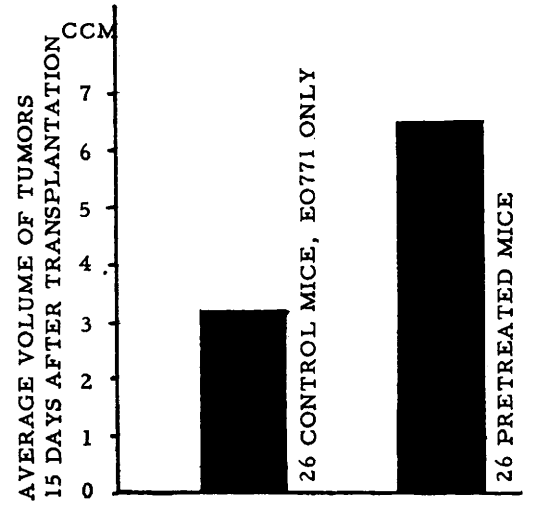


FIG. 2. Growth of EO771 mammary carcinoma in 52 C57 BL/6 mice, 26 of which had been pretreated with non-viable EO771 tumor tissue which had been kept frozen 0°F for 49 days (all 52 mice eventually died of the tumor).

BL/6 mice from the Jackson Memorial Laboratory were inoculated with EO771 mammary carcinoma tissue carried in C57 black/6 mice from the same laboratory. As expected all 52 died from the growth of the neoplasm. However, 26 of the mice which had been pretreated with frozen non-viable EO771 tumor (xyz factor) from the Jackson Laboratory developed a significantly increased rate of tumor growth and earlier mortality than the 26 controls not so treated. The effectiveness for transplanted tumors of the xyz factors in the inbred strain of origin, as demonstrated above, suggests the possibility that spontaneous tumors could be enhanced in the host of origin by xyz factors if such were present in them.

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## A Microbiological Assay for Isonicotinyl Hydrazine. (19709)

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Two essentially different chemical methods have been described for the determination of isonicotinyl hydrazine in biological fluids. The method of Kelly and Poet(1) is dependent on the reaction of the hydrazine portion of the drug with paradimethylaminobenzaldehyde. In the procedure of Rubin *et al.* (2), isonicotinyl hydrazine is decomposed quantitatively into isonicotinic acid and the latter compound is determined by a method employing cyanogen bromide. Since neither chemical assay is specific for the unaltered drug, it was thought that a microbiological method, having a somewhat higher degree of specificity, would be desirable. In the presently described assay, isonicotinic acid is completely inactive and hydrazine has only 1/50 the activity of isonicotinyl hydrazine.

*Experimental. Preparation of the assay plates.* The test organism employed is a strain of *Mycobacterium butyricum,* obtained from the ATCC in 1943 as their culture No. 362. Stock cultures of the organism are maintained on slants of the following composition:

| | g |
|---|---|
| Glucose | 5 |
| Bacto peptone | 5 |
| " yeast extract | 5 |
| " beef " | 5 |
| " agar | 17.5 |
| Distilled water to make 1 liter | |

The pH is adjusted to 6.8 before sterilization. Abundant growth is obtained after 24 hours incubation at 37°C. Stock slants may be refrigerated for several months without impairing their usefulness. Inoculum for seeding the assay agar is prepared in a medium of the following composition:

| | g |
|---|---|
| Bacto casamino acids | 6 |
| Sodium acetate | 1 |
| Trigamine‖ | .45 |
| *dl*-tryptophane | .20 |
| Magnesium sulfate hepta hydrate | .10 |
| Dipotassium phosphate | .10 |
| Calcium nitrate | .08 |
| Ferric chloride, hexahydrate | 1.25 mg |
| Copper chloride dihydrate | 1 mg |
| Manganese chloride hexahydrate | .05 mg |
| Zinc chloride | .05 mg |
| Glucose (added aseptically after sterilization) | 10 g |
| Distilled water to make 900 ml | |

The pH of the medium is adjusted to 6.8 before sterilization and 18 ml amounts are portioned into 125 ml Erlenmeyer flasks. Sterilization is effected at 120°C for 15 minutes. When cool, 2 ml of sterile 10% glucose solution are then added to each flask. For inoculation of the liquid medium a liberal quantity (about one-fourth of a 3 mm loop full) of growth is removed from a stock slant and suspended evenly in the medium. The inoculum is ready for use after 18-24 hours of incubation at 37°C.

The agar used for pouring the assay plates has the same composition as the liquid inoculum medium except for the inclusion of 1.5% agar. Five ml of this medium are poured into Corning pressed flat-bottom culture plates No. 3162 and allowed to harden. Four ml of the same medium, seeded with 3% v/v of 18-24 hour inoculum, are then carefully and evenly layered over the cooled 5 ml of un-

* Contribution 318.

‖ Obtained from Glyco Products Co., Brooklyn, N. Y.