TABLE 1

ULTRAVIOLET ABSORPTION OF THE PRIMARY BAND OF PHENOL IN 95% ETHANOL (READINGS IN 0.020-CM CELLS)

Phenol conc (M/liter)	λmax (mμ)	log ε max
0.000702	218.0	3.782
.00143	"	3.779
.00230	"	3.770
.00366	"	3.769
.00428	218.5	3.772
.00545	"	3.772
.00757	"	3.760
.01085	"	3.752
.01354	"	3.757
0.0168	"	3.739

mum at 272.5 mμ was unchanged beyond that to be expected with modification in solution density (*3*) and was not perceptibly shifted in wavelength. A repeat study on a second series of solutions yielded comparable results.

In view of the discrepancy in the response of the primary band, a series of determinations was made on phenol using water as the solvent. It has better transmission characteristics than ethanol in the lower wavelength region, and observations can be made at sensitivity settings nearer the clockwise limit, in spite of a shift of the maximum of the phenol primary band to about 210 mμ in this solvent. Six consecutive readings over the concentration range 0.0000458M to 0.000210M gave ε values of 6,060 to 5,700 (log ε 3.783 to 3.756) for the primary band, with an apparent shift of the maximum of perhaps 0.5 mμ. Readings at the 270 mμ secondary maximum over the concentration range 0.0001042M to 0.001026M gave ε values of 1,570 to 1,460 (log ε 3.196 to 3.165), with no shift in wavelength. Comparison of these results with those obtained in ethanol would seem to indicate that adequate solvent transmission is a necessary requisite to accurate observation in the lower wavelength region.

Accordingly, solutions of phenol in 95% ethanol were prepared over the instrument density range and read in absorption cells of 0.020-cm light path. The small light path with these cells causes a substantial reduction in solvent absorption and permits readings with 95% ethanol to approximately 202 mμ at a slit width of 1.0 mm. Table 1 gives concentrations and data for the primary band. It is apparent that only small variations in extinction coefficient and wavelength result over a fairly wide range of solute concentration.

The differences in absorption obtained when solutions of phenol are read in cells of standard (1-cm) dimensions as compared with cells of small light path reflect the ratio of the intensity of the stray (not monochromatized) radiation to that of the monochromatized radiation. When solvent transmission is low, so that instrument capacities are strained, the stray radiation becomes a significant part of the total available to the phototube and a drop in observed solution density results. For example, if $I_o = I_m + I_s = 100\%$, where I_m is the monochromatized and I_s the stray radiation, when $I_m = 99\%$ and $I_s = 1\%$ for a solution transmitting actually 40% of I_m, the observed density will be $\log \frac{99+1}{0.4\times 99+1} = 0.391$. If I_s rises to 10%, the observed density for the same solution will be $\log \frac{90+10}{0.40\times 90+10} = 0.337$. These considerations are in accord with the conclusions of Saidel, Goldfarb, and Kalt (*4*) given in a recent discussion of false maxima and stray radiation in the lower wavelength region.

The experiments described above show that the wide range in extinction coefficient and wavelength shift observed by Ungnade, Kerr, and Youse for phenol in ethanol at different concentrations can be associated primarily with experimental difficulties in the region of reduced solvent transmission. Under suitable conditions, large variations indicating a failure of Beer's law do not appear.

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Progressive Tumor Resistance in Successive Generations of Inbred Immunized Rats[1]

Paul Myron Aptekman and Margaret Reed Lewis[2]

The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

The influence of heredity on the incidence of cancer has long been the subject of investigation. Considerable evidence has been collected on the predisposition of humans to cancer, based on statistical studies, and the resistance and susceptibility to spontaneous and transplanted neoplasms, obtained from experimental studies in animals (*1–7*).

In earlier studies (*8–10*), it was found that rats of an inbred strain could be immunized against the growth of transplanted tumors by two methods of experimental procedure. The first method utilized subcutaneous injections of an alcoholic extract of tumor tissue for vaccination against tumor growth, after which the resistance of the treated rats was challenged by implantation of a small amount of viable tumor tissue. This method resulted in the development of tumor resistance in about 50% of the treated rats. Inbreeding of vaccinated rats resulted in an increase in response to this method of tumor

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TABLE 1

TUMOR IMMUNITY IN SUCCESSIVE GENERATIONS OF IMMUNIZED RATS*

Generations	No. treated	No. immune	No. not immune	Percentage immune
Response of offspring of three lines of immunized parents				
SA9	14	14	0	100
SA10	68	67	1	99
SA11	9	9	0	100
(13 litters)	91	90	1	99
SB9	11	8	3	73
SB10	23	23	0	100
SB10	31	27	4	87
(9 litters)	65	58	7	90
SC4	18	16	2	89
SC5	19	19	0	100
(5 litters)	37	35	2	95
Totals 27 litters	193	183	10	95
Response of offspring of vaccinated parents				
31 litters	145	126	19	87
Response of offspring of untreated parents				
19 litters	94	47	47	50
3† "	13	6	7	46
22 litters	107	53	54	50

* Two hundred untreated stock inbred rats implanted with challenge grafts of tumor tissue as controls on the viability of the tumor tissue developed tumors; none were resistant.

† Offspring of untreated parents of generations corresponding to last 3 generations of immunized parents.

immunization from 50% of the offspring of non-vaccinated rats to 87% of the offspring of vaccinated parents, as shown in Table 1.

The second method consisted of implantation of tumor grafts into normal rats, and when the grafts had grown to a small size, the rats were subjected to intratumoral injections of tumor extract. This procedure brought about oncolysis accompanied by the development of tumor immunity in 78% of the treated animals (*9*).

The present study is concerned with the influence of continuous inbreeding of rats, immunized in each generation, upon the response of their offspring to immunization either by subcutaneous or by intratumoral injections of the "vaccine"—alcoholic extract of tumor tissue.

All rats used in this study were of the same strain, closely inbred for many generations under the same conditions. A methylcholanthrene-induced sarcoma #231 (*11*), which had originated in a rat of this strain and to which the rats of this strain had proved to be 100% susceptible, was used for transplantation. The details of the preparation of the vaccine and the methods employed to induce tumor immunity are given in earlier publications (*8, 10*). Two deviations, however, were instituted in the present study—namely, only rats that had proved to be immune were used for breeding, and only transplanted tumors were used in the preparation of the tumor extract (vaccine) for subcutaneous treatment.

Three lines of subcutaneously treated rats, SA, SB, and SC, described in earlier studies (*8*) were further inbred through 11, 10, and 5 generations, respectively. The offspring of the immunized parents in each generation were injected subcutaneously with vaccine and 3 weeks later were tested for resistance. Those that proved to be resistant were tested 1–2 months later for tumor immunity. The immune rats were used for breeding the next generation.

Three lines of rats, TA, TB, and TC, immunized by intratumoral injections, were also inbred through successive generations. The offspring of the immunized rats in each generation were implanted with sarcoma #231, the resulting tumors were treated, and the cured rats tested for tumor resistance, and later for immunity (*9, 10*).

Each time treated rats were implanted with tumor tissue, 4–6 untreated control rats were implanted with a portion of the same tumor tissue to test its viability.

Table 1 shows the results obtained by continuation of inbreeding of the 3 inbred lines, SA, SB, and SC, of rats immunized by subcutaneous injections of vaccine. The rats of line SA are now almost 100% responsive to immunization by injection of vaccine. Only 1 of the 91 rats of the 13 litters comprising 3 generations of inbreeding failed to respond to the vaccine.

The rats of line SB show considerable progress in favorable response. Although only 73% of the 11 rats treated in the ninth generation proved to be immune, 100% of the 23 rats comprising 4 of the litters of the tenth generation were immune, and 87% of the 31 rats comprising the other litters of this generation were also found to be immune.

Of the 37 rats comprising 5 litters covering two generations of further inbreeding of the immunized rats of the SC line, only 2 rats—and these 2 were in one of the litters of the fourth generation—failed to develop immunity in response to vaccination. Every one of the rats in the other 4 litters developed tumor immunity following subcutaneous injections of the vaccine.

In previous studies (*8*) it was found that the response to this method of immunization of the offspring of vaccinated parents (87%) was less than that of the offspring of immunized parents (95%). Inbreeding of the rats has apparently not lessened their susceptibility to the growth of the tumor used, for, as is shown in Table 1, only 46% of the offspring of untreated parents, corresponding to the last 3 generations of immunized parents, proved to be immune.

In the studies of the continuation of inbreeding of rats immunized by intratumoral injection of vaccine, 3 lines of intratumorally treated rats, TA, TB, and TC, were inbred through 12, 11, and 6 generations, respectively. The 3 later generations, comprising 47

litters, or a total of 254 rats, which were first implanted with tumor tissue and later treated intratumorally with vaccine, proved to be 100% immune—subsequent implantations of viable tumor tissue failed to grow in every one of the 254 T rats treated in this manner.

In the T rats of the later generations, the rate of growth of the tumor grafts implanted previous to treatment was slower than in the rats of the earlier generations. Furthermore, they responded more favorably to intratumoral injections, in that, whereas in the earlier generations to obtain successful results it was necessary to treat small tumors 4–6 days after implantation, in the later generations the intratumoral treatments could be initiated on the seventh day with successful results. At the same time, fewer injections were required to bring about oncolysis. In some instances, oncolysis took place after only three injections.

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Histochemical Demonstration of Esterases by Production of Indigo[1]

Russell J. Barrnett and Arnold M. Seligman[2]

Departments of Anatomy and Surgery,
Harvard Medical School, and
Yamins Laboratory for Surgical Research,
Beth Israel Hospital, Boston, Massachusetts

Esterolytic enzymes found in animal tissues are capable of splitting a wide variety of carboxylic acid esters (*1*). Among the aliesterases, specificity of action is provided by the size of the fatty acid moiety rather than the hydroxylic residue (*2, 3*). However, there is considerable overlapping in the hydrolytic properties of the carboxylic esterases. A simple ester such as β-naphthyl acetate is hydrolyzed by serum esterase, pancreatic lipase (*3*), serum cholinesterase, and acetylcholinesterase of brain and erythrocytes (*4*). A method for demonstrating esterase histochemically in acetone-fixed tissues was developed using β-naphthyl acetate as a substrate. By coupling the naphthol with a diazonium salt, an azo dye was produced which was deposited at the sites of enzymatic action (*5*). The process of fixation in acetone and embedding in paraffin removed most of the lipoids and inactivated nearly all the cholinesterase, so that the method served well to demonstrate the nonspecific esterases. However, the lipoid-soluble azo dye diffused in the lipoids of fresh-frozen sections, particularly in nervous tissue which contained acetylcholinesterase (*4, 6*).

Because of the poor solubility of indigo blue in both aqueous media and lipoids, it seemed worth while to examine the properties of indoxyl acetate as a possible substrate for esterases. At the pH optimum for esterase activity, indoxyl is rapidly oxidized to indigo blue and precipitated from solution (Fig. 1). Indigo

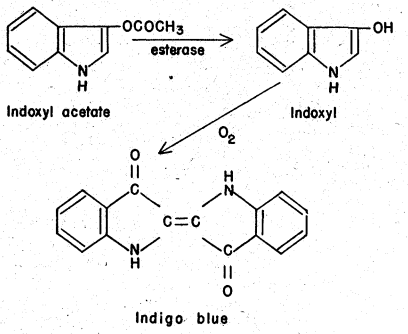

FIG. 1.

blue is a light-fast, stable, brilliant pigment. Indoxyl butyrate was also prepared and used in experiments to test the localization of acetylcholinesterase *vs* other esterases, particularly in nervous tissue.

Indoxyl acetate was prepared in the following manner: A 2-lb tin of sodium indoxyl in a mixed alkali flux, prepared as an intermediate in the industrial production of indigo, was obtained from a commercial source.[3] A portion of the flux was chipped from the solid mass and ground fine. The powder (64 g) was placed in a liter of water and ice in a 2-liter Erlenmeyer flask into which illuminating gas was constantly passed to prevent extensive oxidation. As soon as the melt dissolved, 40 ml 50% (by vol) sulfuric acid was rapidly added and stirred. This was followed by the addition of 25 ml acetic anhydride. The flask was stoppered and shaken. Illuminating gas was bubbled through the mixture vigorously for 10 min. A green-blue precipitate which had formed was collected with the aid of suction and washed with water. The filter cake was extracted six times by suspension in 300 ml boiling water, followed by rapid filtration by the suction pump. A little active charcoal was added during the last few extractions. The filtrates were combined, cooled, and 6–10 g of fine grayish needles was collected. The product was recrystallized from hot water or dilute alcohol in nearly white needles, mp, 126°–128°. The melting point has been reported (*7*) as being 126°–127°.

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