

serum at the end of four weeks at 37° C. produced a strong reaction in dilutions of 1-100 on the scarified rabbit skin and at the end of five weeks it was still moderately active in the same dilution.

When suspended in serum and desiccated *in vacuo* in the frozen state and stored in sealed ampoules at 37° C., only a slight diminution in the titratable potency of the virus takes place over a period of four weeks.

These experiments indicate that normal inactivated rabbit serum, as a suspensoid, serves to maintain the viability of vaccinia virus at temperatures as high as 37° C. over much longer periods of time than do 50 per cent. glycerine or various other substances which we have used. Such a method of preserving the activity of vaccinia is of great practical importance, especially in view of the use of vaccine virus in tropical climates where transportation under unfavorable conditions is necessitated.

These investigations are being carried on further with the purpose of adapting this method to preservation of vaccine for human prophylaxis. The protective action of sera from sources other than the rabbit is also being investigated. The various details of the method will be published at a later date.

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FURTHER OBSERVATIONS ON FACTORS FROM NORMAL TISSUES INFLUENCING THE GROWTH OF TRANSPLANTED CANCER¹

THE presence of an inhibiting factor associated with the transmitting agents of fowl tumor has been demonstrated in the extracts and filtrates of these neoplasms. When properly concentrated it has the property of neutralizing the tumor agent and furthermore will retard the growth of a transplantable mouse sarcoma (Murphy and Sturm). On the basis of these observations, Murphy suggests as a working hypothesis that growth and differentiation of cells is controlled by a balanced system comprising a stimulating force and a retarding force, a suggestion which is in line with other known physiological processes. On the basis of this hypothesis a break in the balanced mechanism leading to uncontrolled growth might take place either by reinforcement of the stimulating force or by suppression of the inhibiting force. Murphy's and Sturm's demonstration that a substance extracted from placenta and embryo skin definitely retards the growth of both transplanted and natural cancers of mice seemed to offer partial support to the general hypothesis. The present report gives further con-

¹ From the Laboratories of the Rockefeller Institute for Medical Research.

firmation in showing two factors in active normal tissues.

The tumor utilized was Bashford Mouse Carcinoma No. 63, and the source of the factors tested was the prelaetating mammary gland of 6 rabbits representing different stages of pregnancy and of one cow at about the fourth month of pregnancy. The fresh tissues were first desiccated *in vacuo* in a freezing box, and the finely ground powder was used in preparation of the extracts. The details of the experiments will be published later, but it should be stated here that they were so designed and controlled as to give each test solution equal representation in the variables associated with tumor growth energy and host susceptibility. The essential point of the procedure was the inoculation of pairs of grafts into each mouse in a series after one graft had been exposed to a test solution concurrently with the exposure of the other graft of the pair to a control solution (Tyrode's solution). For each experiment an additional group of animals was inoculated with two grafts each from the control solution. These data made it possible to analyze the results on the basis of the system developed by Karl Pearson, in particular the Chi square test for "goodness of fit" of frequency distribution.

Significant inhibiting action on the tumor growth was shown in 60 or more cases by the aqueous extracts at pH 7.0-7.3 of the desiccated mammary gland of the six different rabbits. There was no significant difference in the intensity of inhibition between the different rabbits representing a range from 12 to 28 days of pregnancy. The mammary tissue from pregnant mice and from a cow gave equally definite results. The fact that similar extracts of placentas from several of the above animals failed to show any inhibiting action at pH 7.0-7.3² indicates that the results with

TABLE I
TUMOR INHIBITION AND TUMOR STIMULATION AS SHOWN BY
DIFFERENT FRACTIONS OF RABBIT MAMMARY GLAND

Fraction	Concentration Per cent.	Frequency of			N	χ^2	n'	P*
		Inhibition	No effect	Stimulation				
Unfractionated ..	2.6	16	22	4	42	4.06	3	.13
Ether-insoluble ..	1.3	25	16	6	47	6.03	3	.049
Ether-soluble ...	2.0	3	16	25	44	11.05	3	.0040
Double controls .	0.95	12	19	11	42	Standard of reference		
Theoretical normal distribution ...		11	22	11	44	0.21	3	.90

* P = "Probability that deviations as great or greater would occur by chance."

² Definite inhibition was noted when the placenta extracts were used at pH 5.8-6.3. The meaning of this observation is not clear, but it can not be ascribed to the pH, as this is within the range of non-injury to tumor cells.

the mammary tissue can not be attributed to a general foreign substance reaction.

A beginning attempt at fractionation of the desiccated mammary tissue of rabbits 26 days pregnant has yielded a strong tumor-inhibiting ether-insoluble fraction and an equally potent tumor-stimulating ether-soluble fraction. The desiccated mammary gland was first thoroughly extracted with ether and the soluble material, after evaporation of the ether, was suspended in 1 per cent. phosphate buffer. The insoluble portion of the powder was extracted with sterile distilled water. The pH of each test solution was 7.0-7.2.

The results of the tests are shown in Table I and include only those in which all the solutions were tested concurrently on grafts from the same tumor substrate and on mice from the same cage lot. A tumor growth was considered inhibited when the control in the same animal was twice the weight of the test tumor at 21 days or more after inoculation. When the test tumor was twice the weight of the control it was considered as evidence of stimulation. Following the accepted procedure, a significant difference from the normal frequency distribution depends on the value of P being less than .05. The table shows that there was an excellent fit between the frequency distribution of results from double controls and that of the theoretical normal distribution. The results demonstrate not only a definite inhibiting action of the water extract of the ether-insoluble fraction of the rabbit mammary gland, but also an equally definite stimulating property of the ether-soluble fraction from the same tissue. Furthermore, it would appear that there is a partial neutralization of the two forces in the aqueous extract of the unfractionated mammary tissue.

Additional results, including tests of both rabbit and cow glands, are presented in Table II. The results

TABLE II
TUMOR INHIBITION AS SHOWN BY FRACTIONS OF RABBIT AND COW MAMMARY GLAND

Fractions	Frequency of			N	χ^2	n'	P*
	Inhibition	No effect	Stimulation				
Unfractionated ..	154	115	29	298	48.49	3	.000001
Ether-insoluble ..	79	43	9	131	48.02	3	.000001
Double controls ..	63	110	68	241	Standard of reference		
Theoretical normal distribution ...	60	120	60	240	1.01	3	.61

* P = "Probability that deviations as great or greater would occur by chance."

are pooled because there was no significant difference between the effects of extracts of rabbit and cow mammary tissues.

The results seem to offer further support to the idea that active normal tissues may contain two factors, one capable of inhibiting the multiplication of cells and the other augmenting the process.

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DOMINANT LETHAL GENETIC EFFECTS CAUSED BY NEUTRONS

IN crosses of unrelated stocks of the parasitic wasp *Habrobracon*, all females come from fertilized eggs, all males from unfertilized. Treatment of sperm with any physical agent causing dominant lethals should therefore reduce number of female progeny. Number of male progeny should not be affected unless the sperm were themselves rendered incapable of "fertilizing" the eggs. In this case the males would be increased.

As a preliminary experiment wild-type male wasps were sent via air mail to Berkeley, California, and subjected to various dosages of neutrons by Professor Ernest O. Lawrence. Upon being returned these males were crossed with unrelated orange eyed females. Progeny (Table 1) indicate decreased fecundity of the

TABLE 1

Treatment	Total days during which progeny were being produced	Progeny			
		Orange males	Wild-type females	Males per day	Females per day
Control ..	70	42	139	0.60	2.00
530 R. ...	20	14	35	0.70	1.75
900 R. ...	75	43	50	0.57	0.66
1900 R. ..	65	20	5	0.31	0.08

mates of the treated males. It is likely that the fluctuations in number of male offspring are due to small numbers involved.

Although the data presented herewith are meager, they are reported at this time because the cyclotron will not be available for use for several months.

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RECOVERY OF INFLUENZA VIRUS SUSPENDED IN AIR

THE union of two independent techniques has made possible the recovery of the Puerto Rico 8 strain of influenza virus,¹ experimentally suspended in air. One of us (H. W. B.) prepared liquid suspensions of the influenza virus and confirmed, by means of animal inoculation, its recovery from air.^{2,3} The other (W. F. W.) atomized the liquid suspension of virus into

¹ Provided through the kindness of Dr. T. Francis, Jr., Rockefeller Institute.

² H. W. Brown. Unpublished Thesis, Harvard School of Public Health, 1936.

³ H. W. Brown, *Am. Jour. Hyg.*, in press.