imate ratio to each other of 8 : 4 : 2, and considering the evidence presented here that in human cæruloplasmin 4 of the copper atoms are more labile than the rest[10,11], it is tempting to suggest that the observed differences in *p*-phenylenediamine oxidase activity in the plasma of various mammals may be a reflexion of the number of catalytically active copper atoms in the molecule of the cæruloplasmin of each species.

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PROTECTIVE ACTIVITY OF FRACTIONS OF TUBERCLE BACILLI AGAINST ISOLOGOUS TUMOURS IN MICE

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TUBERCLE bacilli and some of their crude fractions have been reported to be capable of initiating a wide range of non-specific changes in the resistance of animals to infectious diseases[1]. Such changes are frequently found to be accompanied by a general enhancement of antibody responsiveness. They have also been shown to be associated with alterations in certain physiological processes believed to be concerned with other aspects of the clearing mechanisms by which micro-organisms as well as foreign, effete, and abnormal cells are removed from the tissues.

It was, therefore, thought of interest to determine whether the moieties of the tubercle bacillus which have proved most effective in eliciting non-specific anti-microbial immunity possess any tumour-inhibitory powers. It has already been reported that intact, living tubercle bacilli of the *BCG* strain retard the progress of several non-isologous cancers in mice[2]. In order to avoid the immunological artificialities inherent in the transplantation of homologous and heterologous tissues, or even of very old isologous ones, all the tumour transplants employed here were of fairly recent, isologous source.

The mice were of the *BALB/c Crgl* and the $C_3H/Crgl$ strains. The tumour tested in the former was a uterine sarcoma (*T*-66); those tested in the C_3H strain were two mammary carcinomas (*T*-32 and *T*-71), a hepatoma (*T*-46), and an osteogenic sarcoma (*T*-62). (The tumours are maintained in the tumour-bank of the Cancer Research Genetics Laboratory of the University of California in Berkeley; the *T* numbers refer to the tumour nomenclature used at the Laboratory.) The tubercle bacillus substances were the following: living *BCG* organisms; intact, phenol-killed and acetone-extracted *BCG*; and several methanolic extracts and their residues, obtained from the phenolized and acetone-treated bacilli. The preparation of these materials has been described elsewhere[3].

The experimental procedure was to subject groups of animals to a single intraperitoneal injection of different quantities of these substances, suspended in 0·5 c.c. saline. Control groups of saline-injected animals were included in every experiment. At different intervals after treatment, isologous tumours were transplanted subcutaneously into the animals by means of a trocar. The transplants consisted of pieces of tumour tissue approximately 0·5 mm.^{3} in size, and were placed on the right flank midway between the axilla and the last rib. Every attempt was made to ensure a maximum degree of sterility in the operation. The development of the tumours was followed by tri-weekly measurements of the two diameters evenly bisecting the palpable growths at right angles to each other.

The results obtained so far can be summarized as follows:

(1) Prophylactic effects of several types and of varying degree were exhibited by all the preparations tested, at certain dose-levels. Several of the non-living preparations were at least as effective as the living *BCG*, and in certain experimental circumstances more so.

(2) Several distinct types of protective effects were seen: an initial retardation of the onset of tumour development, followed by a normal rate of growth; a consistent reduction in the rate of growth; a total prevention of tumour development, with no traces of malignant growth found on autopsy of the animals killed several weeks or months after transplantation; a regression and disappearance of tumours after an initial period of development; an inhibition of metastatic spread; and a prolongation of life of animals which did develop massive tumours despite pre-treatment. Against some tumours only one, and against others several, of these manifestations of heightened resistance were displayed by the preparations.

(3) The dose of the materials was critical, the optimum quantities varying somewhat from one preparation to the next. On the whole, small quantities, of the order of 1·5 mgm., dry weight, or less, were most effective, thus paralleling the results obtained with these materials in protection experiments against heterologous bacterial infections. The optimum doses of the several vaccines differed somewhat *vis-à-vis* the various tumours. Excess quantities sometimes enhanced tumour development, even though the amounts were still considerably below the threshold of gross toxicity.

(4) The protection elicited by some of the preparations was as great, or greater, three months after

Table 1. Protective Effects against a Uterine Sarcoma in *BALB/c* Mice
(One-month vaccination–transplantation interval)

Vaccine	No. of mice out of 7 in which malignant isograft failed to develop*	Tumour area† average values (mm.²) of tumours in those animals which permitted tumour development, at the following intervals after transplantation (days)											
		20	22	25	27	29	32	34	36	40	42	46	48‡
Saline (control)	0	28	40	67	75	88	107	105	122	192	190	198	205
Living *BCG*	2	0	0	60	60	71	68	99	110	119	120	193	233
Phenolized *BCG*, 1 mgm.	4	0	0	25	24	32	50	99	99	99	108	144	156
Methanol extract, Batch No. 1, 1·0 mgm.	0	0	0	24	64	86	115	121	117	197	210	202	252
Methanol extract, Batch No. 2, 1·0 mgm.	3	25	31	56	75	84	96	132	172	166	170	191	247
Residue, 0·5 mgm.	6	24§	36	56	63	63	88	108	120	143	121	240	256
Residue, 1·0 mgm.	0	0	28	104	166	176	216	251	253	383	440‡		

* No tumour found at autopsy 48–50 days after transplantation.
† Product of bisecting diameters.
‡ Tumours grossly necrotic, experiment terminated.
§ Tumour in only one animal of this group.

treatment as it was when only 2–4 weeks elapsed between vaccination and transplantation.

The results of four representative experiments are briefly shown here, in order to illustrate some of these findings:

Experiment 1. Groups of 7 *BALB/c* mice each were injected with one of the following substances: saline; living *BCG* grown for 7 days in liquid culture and then diluted ten-fold; 1·0 mgm. phenolized *BCG*; 1·0 mgm. of a methanolic extract obtained by extracting the organisms for 32 hr. (batch No. 1); 1·0 mgm. of an extract which went into the solvent between the 32nd and 82nd hr. of continuous extraction (batch No. 2); and 0·5 and 1·0 mgm., respectively, of a preparation of the exhaustively extracted residue. All animals received isotransplants of uterine sarcoma *T*-66 one month after vaccination. The number of animals which developed tumours, and the rate of growth of the cancers, are shown in Table 1.

Experiment 2. Groups of 10 C_3H mice each were vaccinated with the same materials described in Experiment 1. Isotransplantation with mammary carcinoma *T*-32 was performed one month later. The transplanted tumours grew in every animal, but differences were observed from group to group in the rate of growth. These are presented in Table 2.

Table 2. Protective Effects against a Mammary Carcinoma in C_3H-Mice
(One-month vaccination–transplantation interval)

Vaccine	Average tumour area* (mm.²) at the following intervals after transplantation (days)						
	11	13	15	18	20	22	25†
Saline (control)	102	130	256	279	369†	—	—
Living *BCG*	32	59	75	119	120	148	207
Phenolized *BCG* 1·0 mgm.	89	122	196	251	319	332	383
Methanol extract Batch No. 1, 1·0 mgm.	32	48	73	167	156	139†	—
Methanol extract Batch No. 2, 1·0 mgm.	90	139	174	241	277	317	402
Residue, 0·5 mgm.	34	52	68	102	112	115†	—
Residue, 1·0 mgm.	62	105	168	225	232	267†	—

* Product of bisecting diameters.
† Tumours grossly necrotic, experiment terminated.

Experiment 3. Groups of 8–10 *BALB/c* mice each were injected with saline; a 1 : 50 dilution of a living 7-day-old liquid medium culture of *BCG*; or 1·5 mgm. phenolized *BCG*. Uterine sarcoma *T*-66 was transplanted into all animals three months later. It was found that the tumours placed into some of the animals regressed spontaneously after an initial period of development, while those of others progressed unchecked. These observations are illustrated in Table 3.

Experiment 4. Groups of 10 C_3H mice each received one of the following preparations: saline; 10-day-old living *BCG*, grown in liquid medium and then diluted 1 : 10; and 0·25 and 0·5 mgm., respectively, of the residue of a methanolic extraction. Four weeks later, isotransplants of osteogenic sarcoma *T*-62 were placed into the animals. The transplants grew progressively in most of the animals, but there were considerable differences in survival-time after transplantation. These are tabulated in Table 4.

These and related findings, as well as the observation that certain fractions of the tubercle bacillus possess the ability of retarding the development of already established cancers in mice, will be reported in detail elsewhere. It might be added that the protective activities of bacillary fractions against malignant tumours may bear some relation to the occasionally reported clinical and laboratory impressions that episodes of certain infectious diseases may have a beneficial influence on already-established cancers, or may reduce the likelihood of then cancer-free individuals developing neoplastic disease after recovery from the infection.

The present findings may also have some relevance for the more fundamental problem of why spontaneously arising tumours are able to grow progressively

Table 3. Protective Effects against a Uterine Sarcoma in *BALB/c* Mice
(Three-months vaccination–transplantation interval)

Vaccine	No. of animals per group	No. of animals with palpable tumours at the following intervals after transplantation (days)										
		4	10	16	22	28	34	40	46	54	60	96
Saline	10	5	9	10	10	10	10	10	10	10	10	All have massive tumours; 5 dead
Living *BCG*	8	6	8	8	7	7	7	7	7	7	7	1 alive, tumour-free; 7 have massive tumours, of which 4 dead
Phenolized *BCG*, 1·5 mgm.	9	4	7	8	8	6	4	3	3	2	2	7 alive, tumour-free; 2 have massive tumours, dead

Table 4. PROTECTIVE EFFECTS AGAINST AN OSTEOGENIC SARCOMA IN C_3H MICE
(Four-weeks vaccination–transplantation interval)

Vaccine	Cumulative number of animals dead* at the following intervals after transplantation (days)						No. of animals free of tumours at autopsy upon termination of experiment on ninetieth day
	45	55	65	75	85	90†	
Saline	0	0	0	2	6	7	1 of 3
Living *BCG*	0	1	3	3	5	7	0 of 3
Residue, 0·25 mgm.	0	0	0	0	2	4	4 of 6
Residue, 0·5 mgm.	0	0	0	0	0	0	3 of 10

* All animals dying before termination of experiment had massive tumours.

† Experiment termination.

in some individuals but not in others. Many aspects of the behaviour of malignant tumours in man and in experimental animals are consistent with the assumption that the ability of malignant cells to grow progressively in the animal body reflects a failure of the antibody or hypersensitivity-forming mechanisms, or of the phagocytic abilities of the reticuloendothelial system[4]. Indeed, the idea has been advanced that the function of the homograft reaction is, primarily, to prevent the colonization of neoplastic cells in the normal individual[5]. Some support for this provocative suggestion comes from the clinical observations that individuals suffering from cancerous diseases show a poor ability to respond with hypersensitivity reactions to unrelated antigenic stimuli[6].

The firm establishment of an immunological theory of progressive malignancy depends, however, on the demonstration that the changes which cells undergo in the transition from normality to malignancy are accompanied by the acquisition of one or more new antigenic determinants. Largely because of the considerable technical difficulties encountered, this has not yet been shown unequivocally. It is possible, nevertheless, to secure further support for the immunological nature of the native defences against neoplastic growth and dissemination by a number of indirect approaches. The results of the present work might be taken to provide some such circumstantial evidence: If progressive neoplastic disease is, in fact, a manifestation of immunological deficiencies, it would be expected that substances which enhance both antibody production and the efficiency of the foreign body clearing reactions will also increase resistance to the development of malignant tumours. It appears that this expectation has been realized in the tumour systems which were the subject of this investigation.

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CHOICE OF ANIMALS FOR BIO-ASSAY

IN marked contrast to earlier views, it is now generally accepted that, in naturally outbreeding organisms, F_1 hybrids between two inbred strains tend to show a lower level of phenotypic variability than do the parental inbred strains. The moral for the bio-assayist in search of uniformity of response, first pointed out by Mather[1], is that F_1 hybrids are, in general, to be preferred to inbreds. We have discussed this point in the course of a review[2] of methods of controlling variation in experimental animals.

The scope of the discussion has now been enlarged by C. K. Chai[3], who rightly points out that the variance of response is often not the only biometrical attribute which is of interest when assessing the suitability of a group of animals for bio-assay. In the case of quantitative (as opposed to all-or-none, or quantal) responses, the precision of the estimates is dependent not only on the variance, s^2, of the responses at a given dose, but also on the slope, b, of the dose-response line, which measures what Chai terms the 'sensitivity' of the animals.

This point was given quantitative expression thirty years ago by the introduction of λ, defined as s/b, as a suitable criterion for the comparison of the economic worth of various quantitative assay techniques for the estimation of a given substance. Thus for this type of assay it is not the absolute values of s and b which concern us, but their relative magnitude. The smaller the value of λ the more information will a given number of animals yield.

Chai also expounds the paradoxical, and potentially confusing, point that in 'quantal', or all-or-none assay the slope is merely an inverse reflexion of the variability of response, according to the relation $s^2 = 1/b^2$. It follows that no information on 'sensitivity' can be extracted from a quantal assay, and that the precision of the estimate is inversely related to the absolute value of b^2.

It is unfortunate that in the course of his further discussion of the question, Chai intermittently loses sight of these clear and valuable distinctions. The resulting confusion is such that the reader may gain an impression that the general superiority of F_1 hybrids over inbreds in respect of s^2 is in doubt. Such an impression is not justified by examination of the evidence presented by Chai. This falls into three main categories: (1) the results of a quantitative assay of testosterone, using the weights of seminal vesicles in castrated mice as the index of response; (2) the results of quantal assay of human chorionic gonadotrophin, using the percentage of immature