to those which develop in conditioned medium. By quantitative criteria also (percentage of muscle colonies which develop) the use of collagen substratum replaces the requirement for conditioned medium.

The implication of these results is that the development of muscle cells requires the presence of a metabolic product of an associated cell type—the fibroblast. The development of a number of different cell types has been shown to be dependent upon the close proximity of connective tissue elements. It is not unlikely that collagen plays a common role in a variety of differentiative events.

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* Predoctoral fellow of the U.S. Public Health Service.

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*INDIVIDUALITY IN NUTRITION: EFFECTS OF VITAMIN A-DEFICIENT AND OTHER DEFICIENT DIETS ON EXPERIMENTAL ANIMALS**

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The genetotrophic principle can be stated in a few words, namely, *the nutritional needs of an organism are determined by the characteristics of the metabolic machinery it has inherited.* This is so obviously valid when applied to different species (e.g., rats, guinea pigs, fruit flies, maize) that it requires no argument. That differences in nutritional needs based upon inheritance also carry over to individuals *within* animal and the human species was postulated by our group in the journal *Lancet* in 1950.[1]

It was further postulated that because of substantial inborn differences in individual nutritional needs, many human disorders of obscure etiology, which character-

istically strike certain individuals and leave others untouched, have roots in the failure of the afflicted individuals to receive continuously or at crucial times completely adequate nutrition in terms of their own peculiar individual needs.

Some conspicuous diseases which may have genetotrophic roots are rheumatoid arthritis, gout, atherosclerosis, dental caries, alcoholism, epilepsy, cataract, acne, mental retardation, deformities at birth, multiple sclerosis, muscular dystrophy, schizophrenia, and mental depression. For each of these (and other) diseases a separate hypothesis should be considered. These individual hypotheses do not necessarily rise and fall together. For example, inadequate individual nutrition during youth might conceivably be found to be largely responsible for the development of teeth which are highly susceptible to infectious decay (animal experiments suggest that this is so), but it might also be concluded, after careful investigation, that nutritional factors have little to do with the development of schizophrenia.

The general concept of genetotrophic disease seems unassailable on logical grounds; each hypothesis relating a particular disease to faulty individual nutrition is either valid or not and is subject to verification—not, however, without difficulty. The seriousness with which these hypotheses will be entertained hinges in part on the magnitude (known or supposed) of the differences in the nutritional needs of so-called normal individuals.

Few investigators would argue against the possibility that nutritional factors may play some slight role in precipitating some of the diseases mentioned, but few, if any, are actively engaged in determining the magnitude of the nutritional factors, presumably partly on the supposition that for the particular disease in which they may be interested, this contribution is slight. If interindividual variations in nutritional needs are substantial, the genetotrophic hypothesis has such tremendous import for human health that medical scientists generally must give it consideration. Particularly is this so because in the case of many of the numerous diseases of obscure etiology no other hypothesis exists to explain their occurrence.

Human experiments are extremely difficult and costly. In order to gain background information that would allow one to assess, *a priori,* the possible prevalence and importance of genetotrophic disease, we have studied the responses of individual weanling animals (in populations not more heterogeneous than human populations) to various deficient diets. The experiments outlined in Table 1 have been performed. These are in three groups: I–III deal with rats and mice on bread and cracker diets; IV–VII involves rats given a diet supposedly otherwise adequate, supplemented with different levels of vitamin A; VIII–XII constitute a repetition and extension of IV–VII for reasons given in the text. These experiments are discussed in order.

Experiment I.—It may be noted that the weight gains of the weanling rats on the enriched bread varied from 2 to 212 gm and that the life spans varied from 6 to 144 days. Translated into human terms this would mean that if a group of 20-lb, year-old babies were fed a comparably deficient diet, some would gain a maximum of 1 lb (5% of their weanling weight) while others would gain 106 lb (530% of their weanling weight). Since it takes an infant about 60 times as long to reach sexual maturity as it does a rat, translation of the life spans into human terms would mean a range of life span for babies on a comparably deficient diet to be about 1–24 years.

The deaths of the animals on the bread diet were associated with different symp-

toms. Though some animals were obviously deficient in vitamin A and showed the characteristic eye symptoms, 22 of the 64 rats died without any eye symptoms whatever. Some few of the rats had highly arched backs (suggesting nerve disorder) before they died. It was evident from outward appearances that the bread diet produced different deficiency states in different individual animals.

Experiment II.—In conversation about experiment I with Professor A. H. Sturdevant, it was suggested that inbred mice may be more uniform in their inheritance than inbred rats, so the experiment was repeated using four inbred strains of mice. In this case the maximum weight gains ranged from 0 (i.e., < 1 gm) to 22 gm, and the life spans ranged from 3 days up to 147 days when the experiment was terminated. At this time 29 of the 58 mice were still alive, and it became evident that the enriched bread diet was less deficient for mice (taken as a group) than for rats. The same very large interindividual differences were observed in the mice as had been observed in experiment I with the rats.

Experiment III.—This is a repetition of experiment II using, however, crackers made from unenriched flour as a sole article of diet. This diet, it was supposed, would be more strikingly deficient. In this case the weight gains varied from 3 to 14 gm and the life spans from 19 to 147+ days (experiment terminated). At termination there were 47 of the 63 mice still living. On the basis of the one criterion of length of life span, the *un*enriched crackers were superior for the mice, as a group, to the enriched bread. Using weight gains and the general appearance of the survivors as a criterion, however, the bread diet was superior to the cracker diet.

Strain differences were very evident in experiments I–III. On the bread diet the water consumption of the Sprague-Dawley and the Wistar rats (as groups) was conspicuously low, especially compared with that of the Holtzman strain. However, the variation within each strain was more conspicuous than the interstrain variation. On the bread diet the Wistar rats as a group performed relatively poorly; the Long-Evans rats had the best weight gains; the longest-lived rats were of the Sprague-Dawley strain. In experiment II all of the DBA mice were dead after 107 days on the bread diet, but about two thirds of the mice of other strains were alive at 147 days, when the experiment was terminated. Incidentally, it was noted that 10 of the 16 DBA mice were alive after 107 days on the cracker diet (experiment III), whereas they had all died by this time on the bread diet (experiment II). The DBA mice were unique in showing this strong contrast.

The behavior of groups of animals did not reflect in any case how individuals within the groups would respond. Interindividual variations were high in all the previous and succeeding experiments. Even among the relatively small numbers of animals used, exceptional animals were observed—both those which thrived unusually well on the deficient diets (regardless of their exact makeup) and those which suffered unusual impairment. This parallels the situation in human populations where exceptional individuals may be found who thrive on what may be regarded as poor nutrition, while others fail to thrive although their nutrition may appear excellent.

Experiments IV–VII.—These four experiments involving different levels of vitamin A were carried out simultaneously, and three striking observations were made: (1) The interindividual variations on the four different diets were so large that after 110 days on the diets *selected animals* from each of the four groups (receiv-

ing, respectively, 0, 1, 8, and 64 units of vitamin A per day) were practically indistinguishable from each other with respect to weight and appearance of general health. (2) In spite of the fact that the animals on 64 units of vitamin A per day (as a group) were superior in weight gains and length of life to any others, two animals in this group exhibited eye pathology scores which were among the highest observed in the entire experiment. (The eye pathology scores were based on daily observations with respect to whether the eyes were watery, swollen, bloody, purulent, or opaque in accordance with U.S. Pharmacopoeia criteria in dealing with vitamin A assays. A score of 1 was given for each day a rat had watery or swollen eyes; a score of 2 was given for each day a rat had bloody, purulent, or opaque eyes. The sum of the daily scores constitutes the eye pathology score of any particular animal.) (3) The animals on the vitamin A-free test diet lived longer (as a group) than those receiving 1 unit of vitamin A per day.

Experiments VIII–XII.—These last two observations led us to postulate the probable presence of some toxic derivative of vitamin A in the food as administered in these experiments. We sought to avoid this possibility in the next experiments by taking great care that at all times, including during mixing, the crystalline vitamin A acetate used and the diets to which it had been added were not exposed to air except under refrigeration. In these experiments the group of animals receiving the lowest level of vitamin A supplementation was markedly superior to the group receiving none, suggesting strongly that a toxic factor had been eliminated. Eye pathology was greatly decreased and delayed but the reason for this was not clear-cut, because this was observed in experiment VIII in which the diet was supposed to be the same as in experiment IV. In experiments VIII–XII the same enormous interindividual differences were observed as before, as is evidenced by the tabulated data.

Some limited experiments were carried out to determine the fertility of the male rats on various levels of vitamin A (experiments IV–XII). After 119 days on the diets indicated in IV–VII each of the 35 surviving rats was placed with a nonpregnant proved female of the same strain. Five rats of the 20 receiving 64 units of vitamin A per day sired litters (2 Long-Evans, 3 Sprague-Dawley). All of the Holtzman and Wistar rats and others at this or lower levels, 30 in all, failed to do so.

The animals involved in experiments VIII–X were tested similarly for fertility when they had been on the respective diets for 54 days. From experiment VIII, 6 rats out of 21 sired litters, from IX, 9 out of 23 did so, and from X, 6 out of 23. A revealing strain difference was observed. No Wistar rat of the 29 tested (including those in experiments IV–VII above) sired a litter. The litters sired by the other strains were: Holtzman—6, Long-Evans—9, Sprague-Dawley—11.

From these limited reproduction experiments we may draw the following tentative conclusions: (1) The U.S.P. test diet is probably inadequate for reproduction regardless of how much vitamin A is added to it. It certainly appears so for Wistar rats and it seems probable that in long-range experiments it would prove deficient for all strains. (2) The amount of vitamin A required to allow reproduction is substantially higher than necessary to satisfy other criteria. (This is in line with the previous findings of others.) (3) The vitamin-A needs of individual rats for reproduction appear to vary widely since some animals sired litters on a vitamin A-free

TABLE 1

NUTRITIONAL EXPERIMENTS PERFORMED

Diet	Animals	No.	Life span (days) (2 lowest, 2 highest)	Maximum weight gains (gm) (2 lowest, 2 highest)	Water consumption (ml/100 gm rat/day) (2 lowest, 2 highest)
Experiment I					
White enriched bread with crusts removed	Rats ♂ Holtzman*	16	33, 34—69, 75	40, 42—77, 85	14.7, 19.0—44.7, 44.9
	Rats ♂ Sprague-Dawley	16	6, 53—122, 144	2, 64—140, 155	12.3, 12.3—25.0, 27.3
	Rats ♂ Long-Evans	16	16, 20—110, 113	23, 33—186, 212	10.9, 11.3—33.2, 33.6
	Rats ♂ Wistar	16	10, 11—87, 87	10, 10—97, 98	11.4, 13.1—23.6, 27.1
	Summary	64	6, 10—122, 144	2, 10—186, 212	10.9, 11.3—44.7, 44.9
Experiment II					
White enriched bread with crusts removed	Mice ♂ A/HeJ	14	3, 10—(10) 147+	0, 4—17, 18	...
	Mice ♂ C_3H/HeJ	14	27, 41—(9) 147+	1, 3—21, 22	...
	Mice ♂ C57BL/6J	15	27, 133—(10) 147+	8, 15—21, 21	...
	Mice ♂ DBA/2J	15	36, 36—87, 107	4, 4—8, 12	...
	Summary	58	3, 10—(29) 147+	0, 1—21, 22	...
Experiment III					
Oyster crackers (Nabisco) made from unenriched flour	Mice ♂ A/HeJ	16	40, 147+—(14) 147+	3, 3—8, 10	...
	Mice ♂ C_3H/HeJ	17	102, 147+—(15) 147+	4, 5—8, 14	...
	Mice ♂ C57BL/6J	14	19, 39—(9) 147+	3, 3—11, 13	...
	Mice ♂ DBA/2J	16	30, 33—(9) 147+	4, 4—14, 14	...
	Summary	63	19, 30—(47) 147+	3, 3—14, 14	...
					Eye path. score† (60 days on diet) (2 lowest, 2 highest)
Experiment IV					
U.S.P. vitamin A-free test diet (Nutritional Biochemicals)‡	Rats ♂ Holtzman	6	43, 48—65, 73	113, 116—193, 213	2, 7—21, 26
	Rats ♂ Sprague-Dawley	6	71, 71—83, 123	151, 209—226, 254	0,1—12, 25
	Rats ♂ Long-Evans	6	13, 76—108, 182+	18, 181—292, 298	0, 11—19, 26
	Rats ♂ Wistar	6	17, 55—91, 182+	28, 119—148, 153	0, 2—24, 32
	Summary	24	13, 17—(2) (182+)	18, 28—292, 298	0, 0—26, 32
Experiment V					
Basal diet plus 1 unit vitamin A per day per rat	Rats ♂ Holtzman	6	6, 28—46, 70	0, 73—134, 195	0, 0—4, 12
	Rats ♂ Sprague-Dawley	6	61, 84—108, 141	144, 168—270, 293	0, 1—10, 83
	Rats ♂ Long-Evans	6	82, 108—134, 135	223, 232—315, 398	2, 10—17, 29
	Rats ♂ Wistar	6	17, 29—132, 132	50, 72—185, 197	0, 0—16, 30
	Summary	24	6, 17—135, 141	0, 50—315, 398	0, 0—30, 83
Experiment VI					
Basal diet plus 8 units vitamin A per day per rat	Rats ♂ Holtzman	6	85, 119—182+, 182+	185, 223—298, 355	0, 0—2, 6
	Rats ♂ Sprague-Dawley	6	69, 106—182+, 182+	179, 196—258, 279	0, 3—9, 21
	Rats ♂ Long-Evans	6	101, 182+—182+, 182+	244, 300—361, 381	4, 5—9, 15
	Rats ♂ Wistar	6	10, 76—182+, 182+	17, 135—295, 295	0, 0—3, 23
	Summary	24	10, 69—12 (182+)	17, 135—361, 381	0, 0—21, 23

Diet	Animals	No.	Life span (days) (2 lowest, 2 highest)	Maximum weight gains (gm) (2 lowest, 2 highest)	Eye path. score† (60 days on diet) (2 lowest, 2 highest)
Experiment VII					
Basal diet plus 64 units vitamin A per day per rat	Rats ♂ Holtzman	6	16, 139—182+, 182+	53, 338—406, 429	0, 0—0, 0
	Rats ♂ Sprague-Dawley	6	182+, 182+—182+, 182+	280, 321—393, 429	0, 0—3, 19
	Rats ♂ Long-Evans	6	34, 182+—182+, 182+	84, 376—457, 547	0, 1—13, 15
	Rats ♂ Wistar	6	9, 182+—182+, 182+	10, 313—369, 390	0, 0—51, 77
	Summary	24	9, 16—(20) (182+)	10, 53—457, 547	0, 0—51, 77
Experiment VIII					
Basal diet only	Rats ♂ Holtzman	6	78, 83—102, 103	148, 174—211, 228	0, 0—0, 0
	Rats ♂ Sprague-Dawley	6	11, 80—179, 182+	39, 180—325, 327	0, 0—0, 5
	Rats ♂ Long-Evans	6	54, 59—134, 182+	151, 159—232, 247	0, 0—0, 0
	Rats ♂ Wistar	6	142, 172—182+, 182+	151, 178—254, 255	0, 0—0, 10
	Summary	24	11, 54—(4) 182+	39, 148—325, 327	0, 0—5, 10
Experiment IX					
Basal diet plus 0.4 units vitamin A per gm diet	Rats ♂ Holtzman	6	48, 182+—182+, 182+	152, 308—358, 359	0, 0—0, 0
	Rats ♂ Sprague-Dawley	6	182+, 182+—182+, 182+	382, 399—459, 494	0, 0—0, 0
	Rats ♂ Long-Evans	6	182+, 182+—182+, 182+	309, 339—374, 384	0, 0—0, 0
	Rats ♂ Wistar	6	182+, 182+—182+, 182+	238, 247—275, 293	0, 0—0, 0
	Summary	24	48, 182+—(23) 182+	152, 238—459, 494	0, 0—0, 0
Experiment X					
Basal diet plus 2 units vitamin A per gm diet	Rats ♂ Holtzman	6	54, 182+—182+, 182+	158, 319—365, 371	0, 0—2, 2
	Rats ♂ Sprague-Dawley	6	182+, 182+—182+, 182+	388, 489—509, 526	0, 0—0, 0
	Rats ♂ Long-Evans	6	182+, 182+—182+, 182+	310, 332—392, 439	0, 0—0, 0
	Rats ♂ Wistar	6	78, 182+—182+, 182+	183, 254—275, 276	0, 0—0, 0
	Summary	24	54, 78—(22) 182+	158, 183—509, 526	0, 0—2, 2
Experiment XI					
Basal diet plus 10 units vitamin A per gm diet	Rats ♂ Holtzman	6	74, 171—182+, 182+	252, 280—394, 397	0, 0—0, 0
	Rats ♂ Sprague-Dawley	6	13, 75—182+, 182+	38, 328—486, 494	0, 0—0, 0
	Rats ♂ Long-Evans	6	182+, 182+—182+, 182+	255, 331—367, 405	0, 0—0, 0
	Rats ♂ Wistar	6	182+, 182+—182+, 182+	227, 230—262, 289	0, 0—0, 14
	Summary	24	13, 74—(20) 182+	38, 227—486, 494	0, 0—0, 14
Experiment XII					
Basal diet plus 50 units vitamin A per gm diet	Rats ♂ Holtzman	6	151, 182+—182+, 182+	228, 284—324, 374	0, 0—0, 0
	Rats ♂ Sprague-Dawley	6	182+, 182+—182+, 182+	376, 387—449, 465	0, 0—0, 0
	Rats ♂ Long-Evans	6	182+, 182+—182+, 182+	343, 362—387, 439	0, 0—0, 14
	Rats ♂ Wistar	6	182+, 182+—182+, 182+	214, 226—245, 251	0, 0, 0, 0
	Summary	24	151, 182+—(23) 182+	214, 226—449, 465	0, 0—0, 14

* The Holtzman rats were obtained from the Holtzman Company, Madison, Wisc., the Wistar rats from Albino Farms, Redbank, N.J., the Sprague-Dawley and Long-Evans rats from Simonsen Laboratories, Inc., Gilroy, Calif. All of the mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. As of August 1961, these mice had been brother-sister mated for 69–118 generations.

† See section on *Experiments IV–VII* in text.

‡ The same basal diet was used throughout experiments IV–XII.

diet, while many other comparable animals failed to do so when the diet contained 2 units per gram or when each rat received 64 units per day.

Because of the very large interindividual differences it is not possible, using one or several criteria, to establish what the vitamin A needs of a rat strain are. Much less would it be possible to assess the needs of experimental rats generally. According to the eye pathology criterion, some appear to need no vitamin A for 67–102 days (experiments IV and VIII); others may develop eye pathology as early as 9 days (experiment V, 1 unit level) or 23 days (experiments VI and VII, 8 and 64 unit levels). The possible toxic factor referred to is a serious complication. On the basis of satisfactory weight gains, some rats appear to have sufficient vitamin A at the level of 0.4 unit per gram of diet; on the other hand, some failed to grow comparably at the 10 unit per gram level or even the 50 unit per gram level.

The human need for vitamin A appears fully as uncertain. Mead, Johnson and Co. offered for 13 years (1932–1945) a $15,000 award to anyone who would determine this need. There were no takers and the offer was abandoned.[2] In the "Sheffield Experiment"[3] one individual was kept on a diet substantially free from vitamin A for as long as 22 months without depletion of the plasma vitamin A or deterioration of dark adaptation. At the other end of the scale, there are those who for obscure metabolic reasons, including in some cases faulty absorption, are benefited by high levels—with respect to dark adaptation, skin conditions, and resistance to respiratory ailments. Unfortunately, the crucial biochemical functioning of vitamin A in areas other than vision, including growth, reproduction, tooth and bone development, etc., is not at all understood.

The question of the toxicity of vitamin A needs to be reconsidered in the light of our experiments. In the latter ones (VIII–XII) we attempted to eliminate a supposed extraneous toxic derivative of vitamin A which may be present in pharmaceutical preparations. In this case, the rats on the lowest supplement of vitamin A, as a group, were markedly superior to those receiving none. In experiment XII animals were often getting 1000 units or more of vitamin A per day. This is a very high level, yet weight gains were only slightly impaired. Adjusted on a weight basis to humans, this would be about 140,000 units per day, or 28 times the "recommended allowance." The toxicity of vitamin A at high levels probably varies greatly from animal to animal.

There are many unanswered questions suggested by our experiments. Why animals (or humans) need such highly variable amounts of vitamin A can hardly be answered at present. Poor absorption, differences in storage and retention may enter in, as well as fundamental differences in tissue needs. The lack of knowledge about the roles of vitamin A, aside from vision, is a severe limitation. The ability of vitamin A *acid* to satisfy all the vitamin-A needs of a rat except those involved in vision[4] is an observation which should eventually help solve the puzzle.

Why the U.S.P. test diet will not support reproduction even if supplemented abundantly with vitamin A is unknown. Our experience (and also that of others) is that laboratory animals do not breed satisfactorily unless they are supplied with some fresh vegetables.

An interesting suggestion comes out of our experience with the partial superiority of unenriched crackers as opposed to enriched bread. Does flour enrichment, which is about 25 years old, need to be re-examined and revised? Is lack of balance among

the supplemented vitamins and minerals the cause of the decreased length of life noted when the mice received bread made from *enriched* flour? This effect was very marked in the case of the DBA mice, but was also noticeable with two other strains.

The question of the relationship of our findings to genetics is very interesting. A technical discussion of this lies outside our competence, however, and would detract from the main objective of our experiments. As we have indicated before,[5] there are many observations which suggest that, among rats, possessing about the same gene pool (i.e., being highly inbred) is by no means the equivalent of being identical. There are unknown factors which control differentiation and the extent to which the various differentiated cells proliferate. These may make possible, for example, the development of large or small thyroids and comparable differences with respect to the entire gamut of hormone-producing and other cells, and probably play an important part in producing biochemical individuality.

From the practical standpoint, the acceptance of what appears to us a fact, namely, that individual animals and humans have highly distinctive nutritional needs, seems in some minds to be equivalent to a denial of the applicability of science to nutrition. This extreme view is not an inevitable one. Speaking now in human terms (since human nutrition is of paramount importance), what is needed is the ability to sort people (crudely at first) with respect to their nutritional needs. This may prove difficult of course—more difficult than sorting potatoes or apples—but no one can know how difficult it is before it is tried.[6] The "normal" person, i.e., one whose every nutritional need (about 40 items) is about average, probably does not exist; yet he receives primary attention. It is for him that "recommended allowances" and "minimum daily requirements" are carefully estimated.

A factor which undoubtedly enters into the problem of nutrition, if we accept the importance of individuality, is that of the self-selection of food. It seems highly probable that this is a potent means by which we often—but not always—get what we distinctively need. This "body wisdom" is doubtless fostered by good nutrition and impaired by poor nutrition; thus, a vicious cycle may be started by inadequate nutrition. Self-selection needs to be studied carefully and on an individual basis.

Summary.—The original elementary question to which we sought an answer was this: Will the responses of individual animals to deficient diets justify *a priori* serious entertainment of the hypothesis of genetotrophic disease as applied to human beings? This question has been answered clearly with a far more emphatic affirmative than we had anticipated. It is a *fact* that individual animals have highly distinctive nutritional needs and there is not the slightest suggestion of a reason why these findings do not carry over to human beings. We have briefly discussed a few of the far-reaching implications of our findings for human nutrition. Any student of human disease who fails to take these concepts into account must be content with a restricted and partial view. Group differences between animals of different strains appear substantial but usually less conspicuous than differences within each strain. These interstrain differences may be expected to be paralleled by ethnic group differences in human populations.

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*MEMBRANE PROPERTIES OF LIVING MAMMALIAN CELLS AS STUDIED BY ENZYMATIC HYDROLYSIS OF FLUOROGENIC ESTERS**

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Fluorogenic substrates are compounds which yield fluorescent products after enzymatic modification. Their utility for quantitative measurements of enzymatic activity at the cellular[1] and molecular level[2, 3] has been previously demonstrated. Among this group of substrates certain fluorescein esters of fatty acids appeared to be of particular interest for the study of living mammalian cells, since their hydrolysis resulted in the intracellular accumulation of fluorescein. In this report we provide evidence which indicates that the intracellular retention of fluorescein is dependent on the integrity of the cell membrane.

Materials and Methods.—Unless otherwise specified, all the experiments were performed with the 2B2 cloned subline[4] of the cultured mouse lymphoma, ML-388, which was kindly supplied by Dr. L. A. Herzenberg. Other cell lines included the human strains, HeLa no. 64266, Hep-2 no. 64267, and HESM no. 64269, a diploid fibroblast line isolated from embryonic skin and muscle. These were supplied by Tissue Culture Associates, Oakland, California.

Primary isolates of tissue pieces, or cell suspensions were obtained from chick embryos, newly hatched chicks, and adult mice. Peripheral blood cells from humans, mice, and chicks were also examined. Among primitive eukaryotic organisms, *Tetrahymena pyriformis, Dictyostelium discoideum* (kindly supplied by Drs. D. S. Natchtwey and R. Sussman, respectively), and isolates from pond water were studied.

Monolayer cultures of 2B2 were grown in a modified Eagle's MEM medium[4] with Earle's balanced salt solution (Microbiological Associates). Cells were removed from culture bottles by replacing the growth medium with 1 ml of phosphate buffered saline (PBS)[5] containing 10^{-4} *M* EDTA and 0.05% trypsin and incubating for 10 min at room temperature (23°C).

With the exception of fluorescein diacetate[6] (synthesized in this laboratory) and of fluorescein dibutyrate (Eastman Kodak), all other fluorescein derivatives[7] were prepared in Dr. J. Moffatt's laboratory at the Syntex Institute of Molecular Biology. For most compounds, a stock solution containing 5 mg per ml was made up in acetone and kept in the freezer. Prior to their use, a dilution of $1:10^4$ was prepared in aqueous medium. Solutions containing more than 1 μg per ml tended to flocculate. The appearance of fluorescence after addition of a fluorogenic substrate to cell suspensions or extracts was followed quantitatively at 37° in a thermoregulated Turner fluorometer model 111 using the filters BG-12 and Wratten 2A-12 for the exciting and fluorescent light, respectively. Purified fluorescein[8] was used as a reference standard.

The fluorescence of individual cells was observed under dark field using a Zeiss microscope equipped with a III Z condenser which permitted alternative observations with either dark field, bright field, or phase contrast. The ordinary tungsten lamp provided with the microscope, in conjunction with an interference filter transmitting a broad band (440–480 mμ), was used as the