TABLE I. Incorporation of Carboxyl Carbon of Caproate Into Blood Acetoacetate.

Time of blood withdrawal after admin. of of caproate-1-C^{14}, min.	Acetoacetate, μM/ml of blood	Radioactivity of acetoacetate in presence of 'carrier' as c.p.m. per mM			Ratio of radioactivity Carbons 2,3,4 Carbon 1, avg
		Carbon 1	Carbon 2,3,4	Total acetoacetate	
0	22				
15	166	10500 ± 200	3650 ± 65	14060 ± 210	.34
30	110	3920 ± 80	1330 ± 40	5250 ± 90	.34
60	103	3980 ± 70	770 ± 25	4750 ± 75	.19
90	100	2240 ± 55	650 ± 30	2890 ± 63	.29

which would alter the fermentation in the rumen, greater quantities of butyric or longer chain fatty acids might be produced. If this should occur, the acids produced in the rumen might be a factor in the development of ketosis.

It is of interest that the activity of the acetone portion and the carboxyl group of the acetoacetate is not equal, which points to the possibility that when caproate is administered to the whole animal, the recondensation of two-carbon fragments derived from this fatty acid does not occur in a random fashion. This is in accord with the observations of Crandall *et al.*(8,9), in their experiments on the metabolism of fatty acids by washed rat liver homogenates.

Summary. Caproate 1-C^{14} was administered to a goat. The resulting increase of blood acetoacetate is roughly proportional to the rise in radioactivity of this compound. The specific radioactivity per mole of the carboxyl group of the acetoacetate is larger than that of the acetone portion. This is another indication that two-carbon compounds derived from fatty acid do not condense in a random fashion to form acetoacetate.

8. Crandall, D. I., and Gurin, S., *J. Biol. Chem.*, 1950, v181, 829.

9. Crandall, D. I., Brady, R. O., and Gurin, S., *J. Biol. Chem.*, 1950, v181, 845.

Received December 7, 1950. P.S.E.B.M., 1951, v76.

Effect of Colchicine on Tumor Growth and Tumor Pyrophosphatase. (18402)

L. BLOCH-FRANKENTHAL AND A. BACK.
(Introduced by B. Zondek.)

From the Department of Radiology, Cancer Laboratories, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

A new attempt has recently been made to explain the influence of colchicine on tumors. According to this theory the regression of tumors obtained by injections of colchicine is due to the action of this substance on the endothelial cells of growing capillaries of the tumor. This action was very marked in "highly cellular rapidly growing tumors"(1). This explanation compares the action of colchicine to that of polysaccharides obtained from *Seratia marcenses* [Shear and coworkers (2,3)]. In the strain of tumor with which we worked, we were unable to observe this hemorrhagic action of colchicine, and since we found this tumor to contain a highly active enzyme, capable of hydrolysing sodium pyrophosphate, we deemed it worthwhile to examine the dependence of this enzyme system

1. Amoroso, E. C., *Nature*, 1935, v135, 266.

2. Shear, M. J., and coworkers, *J. Nat. Cancer Inst.*, 1945-46, v6, 89.

3. Ludford, R. J., *Brit. J. Cancer*, 1948, vII, 75.

on the colchicine injections.

In this paper we describe the influence of colchicine on the growth of a benzopyrene-induced sarcoma (tumor HL) and on the pyrophosphatase contained in it.

Methods. (a) *Rats.* We employed albino rats of both sexes weighing 150 to 175 g, bred in our laboratory for more than 7 years through brother-sister cross-mating. The animals were fed Purina Chow and water *ad libitum*.

(b) *Tumor.* The tumor employed in these experiments was a benzopyrene-induced sarcoma obtained in 1936, and transferred from rat to rat by subcutaneous implantation in the dorsal region. Histologically this tumor is a sarcoma of conjunctival origin with a majority of fusiform cells and containing other cells of different forms and dimensions varying from fusiform cells to giant polynuclear cells. The tumor contains very few blood vessels and is of a hard consistency. In the first stage of their development the tumors were designated as "lentil-", "pea-" and "bean"-sized because exact measurements were difficult to carry out. In the following stages the tumor was measured with a caliper. Each growth experiment lasted 6 to 8 weeks, depending on the cachectic state of the control animals. At the end of each experiment the tumors were excised, measured and weighed.

(c) *Colchicine.* We used BDH crist. puriss. Colchicine in a 0.05% solution in distilled water. Each solution was used for no more than a week after preparation and was maintained at 4° C when not in use. The injections were made subcutaneously in the vicinity of the tumor but not into it. In one series of experiments the injections were begun 3 days after the inoculation of the tumor, but in the majority of cases, 5 to 6 days after tumor inoculation. This period is roughly half the time required by a tumor to reach the size of a lentil, when it first becomes readily observable. In some cases the injections were begun at the lentil stage.

(d) *Pyrophosphatase estimation.* Tumor tissue obtained from rats anesthetised with ether was freed as far as possible from foreign matter such as blood, etc. and suspended in water with the aid of a Potter-ground glass homogeniser. The suspension obtained was tested, usually at 2 concentrations, as to its activity on sodium pyrophosphate under the following conditions:

(1) 1 cc tumor suspension + 2.5 cc acetate-veronal buffer (pH-6.8) + 1 cc $MgCl_2$ 1% ($MgCl_2 \cdot 6H_2O$) + 0.2 cc sodium pyrophosphate 1% ($Na_4P_2O_7 \cdot 10H_2O$) + H_2O to a volume of 5 cc.

(2) Same as in (1) with half the concentration of suspension. The mixtures were incubated at 37° C and the course of hydrolysis was followed by estimating the liberated phosphate after stipulated time intervals. Usually two determinations were carried out, one after 30 minutes' incubation and another

TABLE I. Effect of Colchicine Injections on the Tumor Growth in Grams. Colchicine treatment in all series was identical.

Series No.	Rat No.	Wt of tumor in g	Rat No.	Wt of tumor in g
	Colchicine treated rats (experiments)		Untreated rats (controls)	
1	1	.0		
	2	7.4	10	65
	3	.3		
	4	.2	11	105
	5	10.0		
	8	7.5	12	50
2	16	.2		
	18	5.5	19	38.5
	21	9.0		
	23	.1	20	35
3	24	.0		
	25	19.5	34	51
	26	.5		
	28	9.5	35	38.5
	29	31.5		
	31	.3	36	80
	32	13.5		
	33	17.2	39	82
4	38	1.6		
	40	.7		
	43	36.2	47	62.5
	44	1.7		
	45	.0	48	31
	46	.7		
	50	4.8	49	45
	51	2.8		
	54	.0		
5	101	7.2	97	47.5
	102	.1	98	58.5
	103	1.2	99	85
	118	33.0	100	61
	119	.0	123	40
			125	65
			126	65
			127	87

TABLE II. Effect of Colchicine Treatment of Pyrophosphatase Activity of the Tumor.

Rat No.	0.05% colchicine admin., cc	Period of colchicine treatment	Conc. of tumor suspension tested	Pyrophosphate-hydrolysed after 30 min., %	Pyrophosphate-hydrolysed after 120 min., %	Pyrophosphate hydrolysed by control tumor suspension of untreated rat after 30 min., %	Pyrophosphate hydrolysed by control tumor suspension of untreated rat after 120 min., %
108	1.6	7 days	1/10	16	30	100	
106	1.9	13 "	1/50	15		50	
107	1.6	20 "	1/50	14		50	
115	0.9	5 "	1/10	12		100	
132	1.7	12 "	1/20	10	12	100	
135	1	9 "	1/40	4.5	7.2	65	100
157	1.4	12 "	1/40	16.5	34	90	100
164	2.7	20 "	1/40	14	26	73	100
176	1.8	10 "	1/40	5	12.5	100	
178	2	11 "	1/80	11	21	32.6	70.3
180	1.9	10 "	1/40	7.8	17.7	80	100
181	1.9	10 "	1/40	7	20	80	100
175	2.2	10 "	1/40	42		74.5	
183	3.2	16 "	1/40	11	18	100	
148	1.6	14 "	1/40 1/80				
149	1.8	15 "	1/40 1/80				
150	1.8	15 "	1/40 1/80	No depression in pyrophosphatase activity was observed in these cases			
158	1.4	12 "	1/40 1/80				
163	1.6	12 "	1/40 1/80				
165	2.7	20 "	1/40 1/80				
172	2	9 "	1/40 1/80				

after 2 hours. Phosphate determinations were carried out according to the method of Fiske and Subarow as modified by Lohman and Jendrassik(4) with the aid of a "Lumetron" photometer.

Controls of enzyme, alone, were carried out in each case. Pyrophosphate alone did not liberate any phosphate under the conditions of our experiments. The results, (Table III), represent the corrected values of free phosphate found expressed as percent of the total hydrolisable phosphate present in the mixture.

Results and discussion. As may be seen from Fig. 1 and Table I and II, treatment with Colchicine in most cases caused a considerable deceleration in the growth of tumors. Fig. 1 gives a description of samples from 4 series of experiments. The times of the initiation of colchicine injections fell into 3 groups. In Series 1 the injections were begun 4 days after inoculation, in Series 2 and 3, 5 days after inoculation, roughly half the period required by a tumor to reach the size of a lentil. In the 4th series the injections were begun as late as 14 days after inoculation of the tumor, when it had reached the "lentil" stage. No differences in effect were found among these series. The quantity of colchicine and its distribution in time was also varied from series to series, but was equal in all experiments of a single series.

Table I summarizes increases in weight of tumors in colchicine-treated rats as compared with controls. The difference in weight between these 2 groups is clear. It may be added that the weight of the 3 smallest control tumors is approximately equal to the maximum of 3 tumors treated with colchicine, and that the maximum weight of controls is about 3 times that of the treated ones. However, in each series there were some tumors which were relatively resistant to the action of colchicine.

We were able to observe that in many points the reaction of the strain with which we were working was similar to that described previously by Ludford and others. We could not find any case of hemorrhage in our treated tumors, and an assumption that the action of colchicine in the case of this tumor strain may be analogous to that of bacterial polysaccharide is not probable. As to the

4. Lohman, L., and Jendrassik, *Biochem. Z.*, 1926, v178, 424.

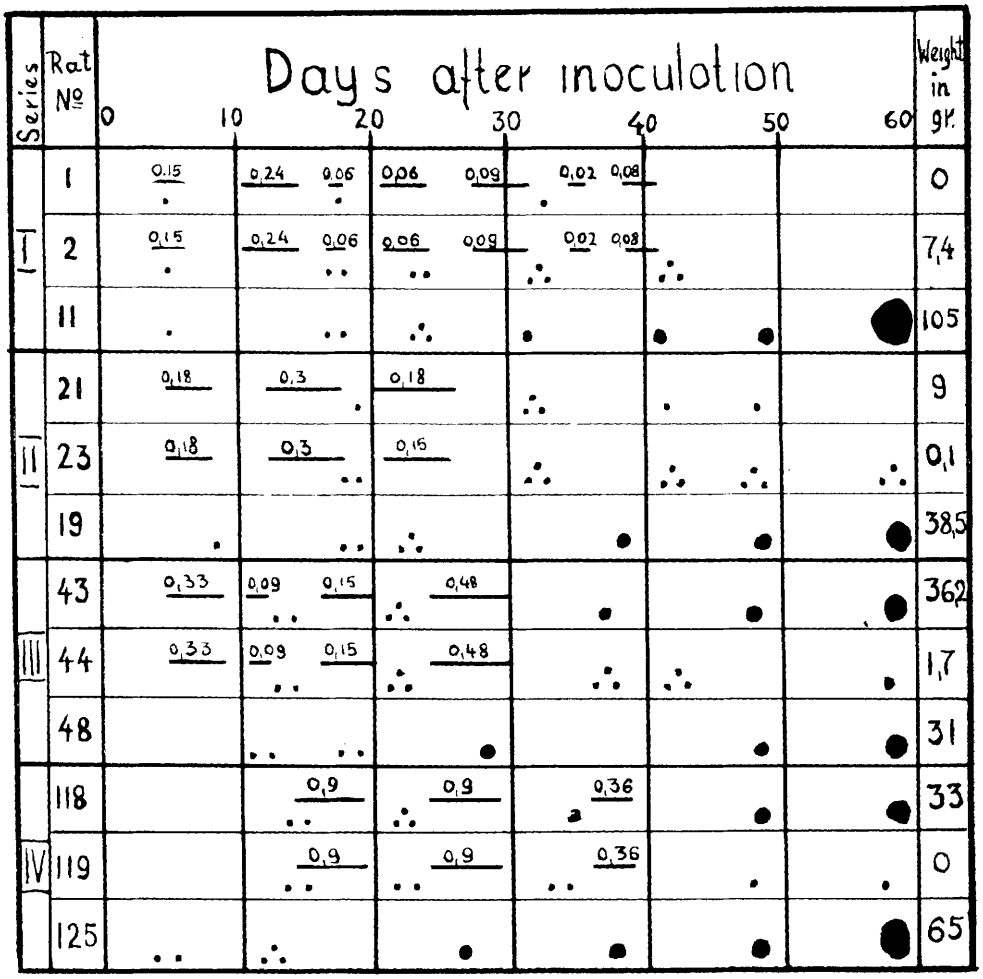

FIG. 1.

The course of some typical experiments from 4 different series on the effect of colchicine on growth. In each series one control and 2 colchicine experiments are presented.

•— lentil size; •• — pea size; ⁘ — bean size.

The denotation of bigger sizes is arbitrary, but each sign is proportional to actual size. Numbers in table denote mgs of colchicine administered. Length of the line under each number is proportional to the number of days this quantity of colchicine was administered.

effect of colchicine on pyrophosphatase, it has already been mentioned that our tumor (HL) was found to contain a potent pyrophosphatatic agent. Its pH optimum was found to be in the range of neutrality in the presence of $MgCl_2$. This salt highly activates the pyrophosphatase, but the tumor displays some activity even without the addition of $MgCl_2$, in contrast to erythrocytes* and brain tissue(6), both of which have about the same pH optimum with regard to pyrophosphate hydrolysis but are almost inactive without added $MgCl_2$.

Among the rat organs tested under the above mentioned conditions (pH 6.8 with added $MgCl_2$) tumor HL was found to be one of the tissues richest in pyrophosphatase. Liver heads the list, and is followed by kidney and tumor HL, both of which are of the

* According to our own experience as well as that of Nagana(5).

5. Nagana, B., and Narayana Menon, K. V., *J. Biol. Chem.*, 1948, v174, 501; 1950, v183, 693.

6. Gordon, J. J., *Nature*, 1949, v164, 579; *Biochem. J.*, 1950, v46, 96.

same order of activity. These are followed by erythrocytes, muscle and dermis in the order mentioned. It should be added that the latter is the mother tissue of tumor HL, but its activity amounts to only about one-fourth to one-third of that of the tumor tissue.

The pyrophosphatase experiments were carried out on a separate batch of rats. Colchicine injections for the purpose of investigating the effect of this drug on the pyrophosphatase activity were administered during the last 2 or 3 weeks before the termination of the experiments. The injections were administered at various stages of tumor growth, and 0.1 mg to 0.15 mg were given daily as often as the rats' state of health permitted. Colchicine injections generally induced diarrhea. On the average the total dose they received amounted to 1 to 1.5 mg.

It should also be mentioned that a high pyrophosphatatic activity was found only in healthy tumor tissue, and that necrotic tissue is practically inactive.

Colchicine has no effect whatever on the pyrophosphatatic activity of tumor tissue *in vitro,* when added to the reaction mixture itself or when incubated with the tissue prior to the addition of the other ingredients. Previous incubation of the tissue causes a decrease in activity, but this is not influenced by the presence of colchicine.

Colchicine *in vivo, i.e.* colchicine administered to the tumor-bearing rat subcutaneously in the vicinity of the tumor for some period of time, had an inhibiting effect on the pyrophosphatase activity in most of the cases tested (Table II). This effect could not be observed in any of the other tissues tested, namely liver, kidney, muscle or skin. In the case of the last 2 tissues the material was taken from the area in which colchicine injections were administered†. The effect does not seem to be a local one.

An acceleration is observed in erythrocytes after colchicine injections, but this is due to the higher erythrocyte concentration in the blood of rats treated with colchicine, caused by the diarrhea and loss of water. On adjusting the erythrocyte concentration of the blood to equal that of the control, equal activities were obtained with normal and colchicine treated rats.‡

In conclusion it may be added that since on the one hand colchicine inhibits the growth of tumor HL in which case no hemorrhage was observed and reduces one of its enzyme activities, and since the rats acquire some tolerance to colchicine after having undergone the first few injections according to our own observations and to those of Ludford, the effect of the latter should further be sought among the enzymatic systems even though the effect on pyrophosphatase does not seem to be the initial one.

Summary. 1. Treatment of a benzopyrene-induced rat sarcoma with colchicine produced tumor regression. This regression was complete in some cases. 2. No hemorrhage has been observed in tumors treated with colchicine. 3. This tumor contains a potent enzyme capable of hydrolysing sodium pyrophosphate. 4. The pyrophosphatase of tumors treated with colchicine is thereby lowered. 5. Colchicine has no effect on tumor pyrophosphatase *in vitro.*

† V. S. Waravdekar and J. Leiter observed an analogous effect with peltatins which reduced the oxydase of mouse sarcome 37 whereas no such effect was observed in the liver, spleen or kidneys(7).

7. Waravdekar, W. S., and Leiter, J., *Cancer Research,* 1949, v9, 625.

‡ Colchicine was injected intravenously for the experiments with erythrocytes.

Received December 11, 1950. P.S.E.B.M., 1951, v76.