

siano a carico del nucleolo, quell'organite cioè che, in base a precedenti ricerche^{1,2,4}, era stato indicato come il primo responsabile della cancerogenesi. Di interesse sarebbero pertanto altre ricerche dirette ad accertare se con virus certamente oncogeni le reazioni del nucleolo possano essere della stessa natura.

Summary. The authors found several modifications, particularly in the nucleolus, in kidney epithelial cells of *Macaca mulatta*, kept in culture for 6-8 days, after infection with polio and Cocksackie viruses. They observed, after infection with polio virus, some groups of virus-like

bodies, which indicate the possibility that a provocation took place in it.

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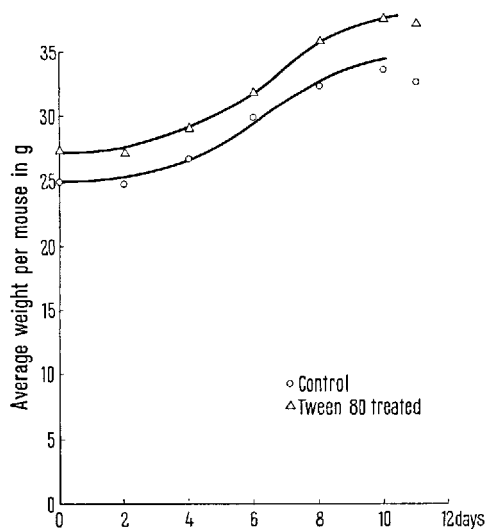
Effects of Tween 80 on the Growth of the Ehrlich-Lettré Ascites Carcinoma¹

It has been shown² that treatment of cells of the Ehrlich Lettré ascites carcinoma by Tween 80 produced marked permeability changes, and associated metabolic alterations as a result of this. During the course of this study it was of interest to observe in preliminary experiments that when the treated cells were inoculated into host mice, the growth of the tumor as measured by weight increase alone, was similar to the normal untreated tumor. In order to verify this observation a study has been made of the growth of this tumor after Tween 80 treatment, with CFW strain mice acting as host for transplantation of this tumor. The results will indicate identical growth patterns thus confirming the earlier preliminary observations on the negative effects of this surface active agent on growth potential of the tumor.

All experiments were carried out with the Ehrlich Lettré hyperdiploid strain ascites carcinoma which has been carried in this laboratory for some years. The tumor was maintained by making serial transplants of 0.2 ml of the ascitic fluid containing cells into female CFW mice of 6-7 weeks of age. The transplants were done between 7-10 days of tumor growth at which time the growth rate is maximal. Fluid for the experiments was drained from several anesthetized mice and pooled. The fluid was divided into two portions of 10 ml each and centrifuged in a clinical centrifuge for 5 min operating at 3000 rpm. The clear ascitic plasma was decanted and saved separately. To the control was added 10 ml of 0.25 M sucrose. The other experimental tube had 10 ml of 0.25 M sucrose containing 1% Tween 80 added to it. Both tubes were stoppered and shaken to mix the cells and fluids thoroughly. After 15 min at room temperature the tubes were centrifuged, as above, at 3000 rpm for 5 min and the supernatant sucrose solutions decanted and discarded. Each tube containing cells was then mixed thoroughly with 10 ml of 0.9% saline and recentrifuged at the above speed of 3000 rpm for 5 min. This washing operation was repeated three times at room temperature, and the supernatant saline wash solution was discarded. To each of the tubes containing the packed cells the appropriate plasma sample saved from above was added, and after stoppering the tubes were shaken to mix the cells. These suspensions of cells were then injected into host mice. For this procedure two groups of 5 mice each were used. The mice were females of about 6-7 weeks of age of the CFW strain.

Injection of control and Tween treated cells was done by intraperitoneal injection of 0.2 ml of the cell suspension. The cell counts of these injections were done using a bright line hemocytometer and using a red cell diluting and counting pipet, with a dilution of 1:100. The number of cells injected in each case into each of the five mice used as controls, and the Tween 80 treated cells, was as follows: $27 \cdot 10^6$ cells control, $24 \cdot 10^6$ cells Tween 80 treated.

During the course of the next 10 days, weight records were made for these two groups of mice. The Figure shows that during the course of the experiment the growth rate was similar in the control and Tween 80 treated cells. At the eleventh day the mice were sacrificed and the fluid was drained carefully into separate 15 ml conical centrifuge tubes. The volumes of total fluid was noted and the tubes were centrifuged at 3000 rpm for 5 min in a clinical



Fluid volume and packed cell volume of control and Tween 80 treated cells.

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² E. R. M. KAY, *Cancer Res.* 25, 764 (1965).

centrifuge, as above. The volume of packed cells was noted in each case. It can be seen in the Table that the fluid volumes were very similar in the control group. The Tween 80 treated group showed a wide variation in total fluid. In the control group the packed cell volume was fairly uniform, while the treated cells did show a variation. The average ratio of total fluid to cells, for all the tubes was 0.26 for the control group, and 0.21 for the treated cells. The cell counts were done by combining all the control tubes and mixing these thoroughly and similarly combining and mixing the Tween 80 treated cells. Cell counts were done as above using a red cell diluting pipet and a bright line hemocytometric slide. The results of these

Vol. fluid ml	Vol. packed cells ml	Cells/fluid ratio	Cell count Day 0	Day 11
Control				
10.2	2.8			
10.2	2.6			
12.3	3.1			
12.0	3.0			
10.5	3.2			
11.0 Av.	2.9 Av.	0.26	$27 \cdot 10^6$	$23 \cdot 10^6$
Tween 80 treatment				
9.1	2.2			
14.2	3.5			
9.0	2.8			
7.0	2.9			
4.8	1.0			
8.8 Av.	2.5 Av.	0.21	$24 \cdot 10^6$	$25 \cdot 10^6$

Cell counts refer in each case to injected number at day 0, and comparable count in harvested fluid at day 11.

counts showed in the pooled control sample $116 \cdot 10^6$ cells per ml. In the pooled Tween 80 treated cells there were $126 \cdot 10^6$ cells per ml. Thus, if the volume used for injection initially, was calculated on the basis of 0.2 ml the number of cells present in the control was $23 \cdot 10^6$ cells per 0.2 ml, and in the treated cells $25 \cdot 10^6$ cells per 0.2 ml. The counts were confirmed with the use of the Coulter cell counter. This also showed that the size class of cells was identical in each case.

It can be seen that the growth of the Ehrlich ascites carcinoma cells in host mice after Tween 80 treatment appears to be normal. It is not possible to explain the variation in ascites fluid volume of the individual mice after injection with the treated cells. It is probable that this is related in some way with the variation in cellular infiltration into the tissues during early stages of growth of the tumor. It seems evident, however, that treatment of the cells with Tween 80 does not appreciably alter their growth potential. In the earlier findings² it was suggested that the cells are able to restore the altered membrane constitution affected by Tween 80 treatment by metabolic processes during the recovery phase in the growth conditions in host mice. These experiments would tend to confirm this.

Zusammenfassung. Der Einfluss von Tween 80 auf das Wachstum des Ehrlich-Lettré-Carcinoms war statistisch nicht zu sichern, wenn die Gewichtszunahme allein gemessen wurde. Individuelle Variation im Flüssigkeits- und Zellgehalt zeigten, dass oberflächenspannungs-herabsetzende Substanzen das Carcinomwachstum durch Eigenschaftsänderung der Zellmembran beeinflussen können.

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Transport of Thiamine by the Small Intestine in vitro

In man, the intestinal absorption of thiamine presents a definite obstacle¹, since single oral doses above 2.5–5 mg greatly increase the faecal excretion^{2–4}. The results of DA SILVA and IVY⁵, in dogs with a chronic Thiry fistula, support this view. In rats, simple diffusion seems to regulate the intestinal absorption of thiamine in vivo^{6,7}, at least when relatively high doses of vitamin are used. However, VENTURA et al.⁸ showed comparatively greater absorption of a small than of a high dose of thiamine, and POLIN et al.^{9,10}, employing intestinal loops of chicks in situ and the antithiamine Amprolum, suggested that the thiamine uptake may be an active process superimposed on some passive absorption.

By the everted-intestinal-sac technique, TURNER and HUGHES¹¹ were unable to find in vitro any evidence of an uphill transport using $20 \mu M/l$ concentration of thiamine. However, since with several substrates (e.g. basic amino acids¹², pyrimidines¹³, *d*-xylose¹⁴) the demonstration of an uphill intestinal transport could be achieved only by using very low initial concentrations, we applied

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