selverschiebung geht anscheinend mit dem Grad der Malignität einher. Der Ausfall oxydativer Energiegewinnung wird rein quantitativ wohl durch die Erhöhung der glykolytischen Milchsäurebildung kompensiert. WARBURG[2] aber bezeichnete die glykolytisch gewonnene Energie als morphologisch minderwertig. Auch der Verfasser geht in den nachstehend geschilderten Experimenten von der Idee aus, es könnte ein «Qualitätsunterschied» von glykolytischer (plasmatischer) und oxydativer (mitochondrialer) Energiegewinnung vorliegen, woraus eventuell ein Energiedefizit in der Tumorzelle resultiert.

Aale, die Hauttumoren aufwiesen, wurden mehrere Wochen lang zunächst mit niedrigen Konzentrationen (1 mM/l) von anorganischem Diphosphat ($Na_4P_2O_7$, PPi) behandelt, das nach BALTSCHEFFSKY[3] wie ATP und andere organische Phosphate in der Zelle als Energiedonator fungiert. Das PPi wurde dabei lediglich dem Hälterungswasser zugesetzt. Nach 3- bis 6wöchiger Behandlung ist bei einem Teil der Tumoren ein Ansatz zu vermehrter Schleimzellenbildung zu beobachten. In seltenen Fällen haben sich sogar Nester von dichtgedrängten, wenn auch relativ kleinen Schleimzellen entwickelt; in unbehandelten Tumoren treten Schleimzellen lediglich vereinzelt auf. Wenn sich dieser Befund in weiteren Versuchen bestätigen lässt, könnte man das als eine erhöhte Differenzierungsleistung des Tumorgewebes durch zusätzliche Energieversorgung deuten.

In parallel laufenden Experimenten wurde die PPi-Konzentration schrittweise jeweils im Abstand mehrerer Tage von 1 mM/l bis maximal 4 mM/l gesteigert. Dabei kommt es manchmal schon nach einer ersten Konzentrationssteigerung auf 2 mM/l innerhalb von 1 bis 3 Tagen zu einer Zerstörung des Tumors. Die Tumoren schilfern Stück für Stück ab, kleinere Tumoren (bis 10 mm ⌀) werden auch als Ganzes abgestossen. Besonders bei grösseren Tumoren zeigt sich zuvor eine gelatinöse Verquellung der Tumoroberfläche als Ausdruck einer Verflüssigung des Gewebes (Kolliquationsnekrose). In der entstehenden Wunde bleiben meist einige Reste epidermalen Tumorgewebes, vom Bindegewebe umsponnen und festgehalten, zurück. Blasig aufgetriebene Zellkerne und Lockerung des Gewebeverbandes weisen auf den angegriffenen Zustand dieser epidermalen Tumorreste hin. Das spätere Narbengewebe neigt zu einer Erneuerung des Tumors (Rezidivbildung).

Im bindegewebigen Stroma sowie im benachbarten Bindegewebe hat sich besonders während der Zerstörung des Tumors ein zellenreiches Material angesammelt. Hierzu gehören vorwiegend kleine Lymphozyten, Makrophagen und indifferente Bindegewebskörper. Das epidermale Tumorgewebe ist von kleinen Lymphozyten reichlich infiltriert.

Drei Interpretationsmöglichkeiten bieten sich für die Abstossung der Tumoren durch PPi-Einwirkung an. 1. Die zusätzliche Energiezufuhr erhöht die Differenzierungsleistung der Tumorzelle und damit die Ausprägung von Oberflächenstrukturen, auch von tumorspezifischen Antigenen, sogenannten Neoantigenen. Daraus resultiert eine intensive Immunreaktion, die zur Zerstörung des Tumors führt. 2. PPi wirkt als Adjuvans, es kommt also zu einer allgemeinen Aktivitätsverstärkung des immunkompetenten Systems. 3. Höhere Konzentrationen von PPi wirken sich toxisch aus, wobei Tumorgewebe empfindlicher reagiert als normales Gewebe. Die Anreicherung immunkompetenter Zellen wäre dann nicht Ursache, sondern Folge der Tumorzerstörung.

Summary. Eels with dermal tumors, consisting mainly of undifferentiated epidermal cells, have been treated with inorganic diphosphate. Depending on concentration of the diphosphate used, the tumors show either an increased number of mucous cells or they are repelled. In the adjacent tissue of the cutis, during these processes, a great number of lymphocytes and macrophages are found. Three possibilities are offered to explain these facts, the most important of which is that the diphosphate increases the ability of the tumor cells to redifferentiate. Consequently, formation of tumor specific antigens is induced, as a result of which the tumor is destroyed by an immunbiological reaction.

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The Destruction of Lymphoma Cells by Activated Human Lymphocytes

Human lymphocytes are mitotically activated by culture with leucocytes of another donor[1]. The degree of activation has been shown to be completely dependent upon HL-A determined incompatibility[2]. The intensity of the stimulation is also dependent on the nature and metabolic state of the cell on which the antigen is presented. Thus viable fibroblasts are ineffective as stimulant cells despite the fact that HL-A antigens have been shown to be present on their surface[3]; lymphoblasts are more effective as stimulant cells than small lymphocytes from which they have been derived[4] and disrupted lymphocytes are inactive[3,5]. Of all cell types tested one was outstandingly potent viz. cells from Burkitt lymphoma and other lymphoid cell lines[6]. Transplantation and other antigens appear to be peculiarly effective in stimulating human peripheral lymphocytes when they are presented on metabolically active human lymphoid cells.

Activated human lymphocytes are known to be cytotoxic towards some other human cells (e.g. Chang liver cells) in tissue culture[7]. Cytotoxic capability is a property only of immunocompetent lymphocytes; leukaemic lymphocytes and cells from Burkitt lymphoma cell lines have been shown not to possess this property[8] and it is very probable that cells of all continuously growing lymphoid cell lines lack immunocompetence, whatever their derivation. Immunocompetent cells are not themselves readily destroyed by lymphocytes of another donor; destruction is barely detectable in normal mixed lymphocyte cultures[9]. HOLM[8] could not detect damage to lymphocytes

Table I. Cytotoxic activity of stimulated and unstimulated human lymphocytes on cells from a lymphoid cell line

Source of lymphocytes and No. of cells	Irradiated cells present during pre-incubation	Release of ^{51}Cr from EB2 cells labelled with ^{51}Cr		
		Test	Red cell control	Incubation period (h)
Human cord Blood No. 22 2×10^6 cells	10^5 EB2	53	12	6
	none	32	15	6
	10^5 EB2	63	19	23
	none	42	21	23

Irradiated EB2 cells received 6000 r of X-irradiation. The incubation period before addition of ^{51}Cr-labelled EB2 cells (pre-incubation) was 3 days. During the 24 h period from day 3 to day 4 the ^3H-thymidine incorporation of the stimulated lymphocytes was 38,896 dpm and of unstimulated lymphocytes 5148 dpm. The ^3H-thymidine added was 0.5 µci of specific activity 150 mci/m*M*.

Table II. Cytotoxic action of cord blood lymphocytes activated with various stimulants

Culture medium	Stimulant added	^3H-thymidine incorporation (dpm)	^{51}Cr release from ^{51}Cr-EB2	
			Test	Control
Eagle's medium containing 20% human serum	none	2,795	21.7	16.6
	irradiated EB2 (5×10^5)	14,190	53.2	14.3
	irradiated EB2 (10^5)	37,293	42.9	15.8
	irradiated EB2 (5×10^4)	11,975	24.1	14.7
	PHA (30 µg/ml)	12,626	14.2	15.4
	SF (1 in 10)	7,506	20.3	14.6
Eagle's medium containing 20% foetal Bovine serum	none	6,915	25.5	14.5
	irradiated EB2 (5×10^5)	14,266	47.0	13.6
	irradiated EB2 (10^5)	24,730	42.3	14.4
	irradiated EB2 (5×10^4)	12,960	29.8	14.8
	PHA (30 µg/ml)	8,784	14.9	14.6
	SF (1 in 10)	9,022	25.1	13.9

The phytohaemagglutinin (PHA) was a commercial preparation obtained from Burroughs Wellcome and Co. Staphylococcal filtrate (SF) was a filtrate of a culture of *Staphylococcus aureus* grown in 199 medium[9]. The incubation period for the ^{51}Cr release was 7 h. When set up cultures contained 2×10^6 lymphocytes in 1 ml of medium. Control cultures contained equivalent numbers of red cells but no lymphocytes. ^3H-thymidine measurement as for Table I, but from day 5 to day 6.

Table III. Cytotoxic action of cord blood lymphocytes stimulated with X-irradiated EB2 or Jiyoye cells

Source of lymphocytes and No. of cells	Irradiated cells present during pre-incubation	^3H-thymidine incorporation (dpm)	% ^{51}Cr release when incubated with			
			^{51}Cr-EB2		^{51}Cr-Jiyoye	
			Test	Control	Test	Control
Human Cord blood No. 30 2×10^6 cells	none	5,022	13.6	11.5	14.9	14.3
	10^5 EB2	39,072	29.8	–	25.6	13.2
	10^6 EB2	4,427	36.7	12.1	15.7	13.3
	10^5 Jiyoye	37,550	21.8	12.9	15.5	12.8
	10^6 Jiyoye	32,435	36.0	12.1	21.9	14.0

Incubation period for ^{51}Cr release = 6 h. Cells were cultured for 6 days prior to the addition of ^{51}Cr labelled cells. ^3H-thymidine incorporation as for Table I, but from day 5 to day 6.

exposed to a 100-fold excess of allogeneic lymphocytes for up to 72 h. Since human lymphocytes are so markedly activated by cells from lymphoid cell lines it was thought that they may have a correspondingly marked capacity for destroying these lymphoid cells. The following experiments indicate that this is indeed the case.

Materials and methods. Burkitt lymphoma cell lines EB2 and EB4, obtained from Prof. M. A. EPSTEIN in May 1968, have been routinely maintained in stationary cultures in Eagle's medium containing 20% bovine serum. Irradiated suspensions were prepared by resuspending the cells, after centrifugation, in Eagle's medium containing 20% pooled human serum and exposing to 6000 r of X-rays[6]. Blood lymphocytes suspended in the same medium at 2×10^6 per ml were obtained from defibrinated human adult or cord blood after sedimentation of the majority of the red cells with gelatin[9].

Aliquots of the blood lymphocyte suspensions (1 ml) were cultured in an atmosphere of 5% CO_2 in air for several days in 7.6×1.27 cm round bottom glass tubes with or without the addition of irradiated EB cells. Controls were set up containing the same number of the donor's red cells but no lymphocytes. The cytotoxic potential of the cultured cells was then assessed by a chromium release technique[10]. A volume of 0.5 ml was removed from the culture fluid in each tube taking care not to disturb the cells at the bottom. It was replaced by 0.5 ml of medium containing 10^6 non-irradiated EB cells which had been labelled by incubation with ^{51}Cr-chromate (1 µci per 10^6 cells) for 24 h. After a period of incubation with ^{51}Cr-labelled cells the culture tubes were centrifuged and the ^{51}Cr present in supernate and deposit counted. From the two counts the percentage released was calculated. All results are means of triplicates.

Results and discussion. Lymphocytes which had been stimulated with irradiated EB cells were markedly cytotoxic to cells of the same type (Table I). Unstimulated cells were much less cytotoxic but usually produced a measurable cytotoxic effect. The cytotoxic reaction was a rapid one readily demonstrable over a 4–6 h period and was a first-order reaction with respect to concentration of labelled EB cells. No cytotoxic activity was found in supernates of EB cell-lymphocyte mixtures. Similar characteristics have been reported for other lymphocyte-target cell systems[7,11,12]. The activated lymphocytes probably exert a direct cytotoxic effect on the EB cells. It is unlikely that the effect is significantly augmented by a factor in human serum comparable to that described for Chang liver cells[13]. The cellular cytotoxic reaction

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could be obtained as readily when both the activatory and cytotoxic stages of the reaction were performed in medium containing foetal calf serum instead of human serum (Table II).

It was expected that lymphocytes activated by stimulants other than irradiated EB cells might be cytotoxic to EB cells in view of the reports that lymphocytes activated by a variety of stimulants are cytotoxic to Chang liver cells[7,8]. In practice, lymphocytes stimulated with phytohaemagglutinin or staphylococcal filtrate proved to be much less cytotoxic to EB cells than lymphocytes of the same donor stimulated with irradiated EB cells (Table II). The results may indicate that some specificity of the inducing agent is required. Lymphocytes activated with a high dose (10^6) of irradiated EB cells were more cytotoxic than lymphocytes activated with a low dose (10^5) even though the level of DNA synthesis measured was greater with the smaller dose. However, the specificity seems to be limited. Lymphocytes stimulated with either EB2 cells or Jiyoye cell line cells showed no differential killing effect towards the two cell types (Table III). We are currently investigating the question of specificity employing other cell lines[14].

Résumé. Les lymphocytes frais du sang humain, après culture avec des cellules lymphoïdes de lignées continues exposées aux rayons X, peuvent tuer ces cellules ou celles d'une autre lignée lymphoïde. Les lymphocytes humains stimulés par culture avec la phytohaemagglutinine n'ont pas cet effet.

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The Induction of Congenital Abnormalities in Mice by Means of Heterologous Anti-Mouse Placenta Serum[1]

The production of congenital malformations after the injection of heterologous anti-rat kidney sera into pregnant rats and mice was reported by different authors[2–6]. Similar results were obtained in rats using heterologous anti-rat placenta sera[7,8].

This paper is concerned with the effect of 2 different forms of heterologous anti-placenta sera in mice: against fresh and lyophilized placental tissue and the possible modification of this effect produced by absorption of the antisera with mice blood, always present in the antigenic tissues.

The interest of studying this teratogenic effect in mice is stressed by the observations about the presence of spontaneous congenital malformations in inbred animals[9].

Material and methods. Adult female Swiss albino mice weighing 25–30 g were mated overnight. Vaginal content was checked every morning and the day in which a plug was found was considered as day 0 of pregnancy. Animals were sacrificed on days 14, 16 or 18 and the dissected placentas were weighed. Aliquots of the 3 ages were pooled, homogeneized in an equal volume of saline in a cooled Virtis homogeneizer. A part of this homogenate was used as antigen without further treatment (fresh placenta). Another part was lyophilized and stored in a freezer at − 25 °C. When used, it was reconstituted with distilled water: 1 ml for each 100 mg of powder (lyophilized placenta).

15 New Zealand adults rabbits were used for the preparation of antisera. 6 of them were injected with fresh placenta, 6 with lyophilized placenta, 1 with mice serum, 1 with mice red cells and 1 with mouse whole blood. All rabbits were immunized with intradermic injections of 1 ml of antigen emulsified in equal volumes of Freund's complete adjuvant[10]. Booster injections of antigen alone were given usually at 15 day intervals and by different routes, during a period of 3–6 month. All sera obtained during and after immunization period were stored at − 25 °C.

Testing of antisera was done by immunodiffusion[11] and a passive hemagglutination test[12]. In both techniques, the placental antigen was the same suspension used for sensitization, except that it was centrifuged at 3500*g* for 90 min at 4 °C. The protein concentration in the supernatant was measured according to WADELL[13] and adjusted to the concentration of 10 mg/ml. Antibodies against mouse blood, present in the anti placenta sera were removed after inactivation at 56 °C for 30 min, incubated with heparinized mouse blood (0.2 ml/ml antisera) for 60 min at 37 °C in a water bath with constant agitation, and overnight at 4 °C. The mixture was centrifuged at 2500*g* for 30 min. The supernatant was separated, checked by immunodiffusion in presence of placental antigen and mouse serum and stored at − 25 °C.

5 groups of experiments were performed as shown in the Table. Age of pregnancies was established by the vaginal plug method as described above. Antisera were injected i.v. in the tail. A single injection of 1 ml/100 g body weight was given to each animal. Injected females were maintained in isolation with food and water ad libitum and sacrificed on the 18 th day of pregnancy by exsanguination. Uterine horns were exposed and implantation sites were registred. Fetuses were separated and inspected for external malformations.

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