Douglas *et al.* (*17*), using paleontological arguments, recognize a worldwide rise of Cretaceous sea level that began with a major pulse in the Albian and climaxed near the end of the Turonian. Larson and Pitman (*18*), tracing rates of spreading by magnetic lineations, propose a rapid increase of spreading 110 to 95 million years ago in the Pacific and Atlantic oceans, which would cause a dramatic rise of sea level. Thus, geophysical and paleontological arguments indicate a rapid rise of sea level, starting 110 to 100 million years ago, which we conclude ultimately caused the drowning of the reef faunas of the Mid-Pacific Mountains and the Japanese Seamounts during Albian-Cenomanian times. These drownings are probably part of a larger phenomenon and should correlate with the middle Cretaceous transgressions that exterminated coral-rudist reef communities throughout the world (*17*).

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10. The age of the Albian chalk indicated by the dotted line (Fig. 2) is based on foraminiferans dug from cracks in dredged volcanic rocks; the chalk probably dates from the volcanic inception of the seamount (*4*). The solid line indicates the occurrence of shallow-water fossils encountered during the drilling of hole 171 by the *Glomar Challenger*, Leg 17 (*5*). These fossils must be Cenomanian or older, because Cenomanian chalks overlie them. Cores from Leg 17 show that the chalk was deposited almost without interruption throughout the remainder of the Late Cretaceous. The dotted lines (Fig. 2) are thus shown as segments to differentiate them from the chalks of other guyots, where the length of the dotted line indicates the range of the contained fossils.
11. No Danian or Paleocene chalks were dredged from any of the guyots sampled during the Aries V Expedition. Sediments of this age are absent or only thinly developed elsewhere in the Pacific Ocean (*5*). A lowering of fertility rates and a rise of solution levels may explain this lack of sediment (*5*, *19*).
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Lymphocyte Antigenicity Loss with Retention of Responsiveness

Abstract. *During culturing at 22°C for more than 4 days, human peripheral blood lymphocytes lose their ability to stimulate allogeneic lymphocytes in mixed lymphocyte cultures. The cells retain their ability to respond to allogeneic lymphocytes or phytohemagglutinin for up to 10 days of culturing. The findings are relevant to reports on successful transplantation of cultured skin.*

Lymphocytes respond to alloantigenic differences in vitro by transformation into blast cells (*1*). A curious fact has been that lymphocytes also act as the most effective stimulating cells to induce such transformation. It could be asked whether there is some special relation between the "recognizing" sites and the "recognized" antigens on lymphocytes. We describe here the surprising finding that, upon aging in vitro, lymphocytes lose their ability to stimulate allogeneic cells while they retain their physiological ability to respond. The stimulating antigenic structures may therefore be labile in tissue culture, as has been suggested by the work of Summerlin on cultured skin (*2*).

Peripheral blood lymphocytes containing less than 5 percent granulocytes were separated from heparinized blood of healthy unrelated volunteers by Ficoll-Hypaque gradient centrifugation. Cells were first cultured at 22°C in McCoy's medium for varying numbers of days as indicated below; cell viability after this preliminary culturing (preculture), as determined by trypan blue exclusion, was greater than 80 percent in all instances prior to use in transformation experiments. The cells were adjusted to a concentration of 10^6 per milliliter; cells stained with trypan blue and thus shown to be nonviable were not counted. Mixed lymphocyte culture (MLC) tests were done in triplicate as described by micromethods (*3*)

Table 1. Effect of preliminary culturing of lymphocytes on their responsiveness to PHA. Phytohemagglutinin (25 μg in 0.01 ml volume) was added to 100,000 responding cells in 0.1 ml volume. After 3 days of incubation at 37°C, [^{3}H]thymidine (0.8 μc in 0.02 ml) was added, and the cultures were precipitated after additional incubation overnight. Lymphocytes from 86 persons were tested.

Preculture (days)	Radioactivity [(count/min) ± S.E.]		Ratios in individual experiments
	PHA stimulation	Background	
0	74723 ± 4294	1583 ± 140	75 ± 13
3	68816 ± 5661	619 ± 287	180 ± 49
4	51727 ± 5700	443 ± 69	162 ± 30
6	21205 ± 7540	460 ± 193	63 ± 6
7	25840 ± 3819	254 ± 37	82 ± 11
10	18788 ± 5147	252 ± 123	98 ± 22
16	1485 ± 265	147 ± 35	11 ± 5

in which incorporation of [^{3}H]thymidine is taken as a measure of DNA synthesis of the responding cells. The counts per minute given are the mean counts (± the standard error) of the three parallel cultures. Stimulation ratios were calculated by dividing the average number of counts per minute of a particular experiment by the average of the control (background) culture.

The response to phytohemagglutinin (PHA) of cells precultured for varying numbers of days is given in Table 1. A progressively lower number of counts per minute is obtained with increased time of preculture. Because of a similar drop in control radioactivity, a significant reduction in stimulation ratios is not seen. Although it is uncertain whether the number of counts per minute or stimulation ratio is a more accurate quantitative expression of lymphocyte responsiveness, the cells are shown to be capable of responding to PHA by both methods. This is clear evidence that the lymphocytes were viable after the preliminary culturing.

The loss of antigenicity of precultured cells in MLC is demonstrated in Table 2 and Fig. 1. The two precultured cells A and B respond strongly to fresh cells C and D, but not to each other. They are unable to stimulate the same fresh cells to a significant degree (Table 2). Thus, preculturing has abolished the ability of the cells to stimulate, but not their ability to respond; the opposite effect is produced by treating fresh lymphocytes with mitomycin.

HL-A typing of the cells was performed by the microcytotoxicity method (*4*) before and after preculture, without any change in the numbers or specificities of antigens. Care was taken to exclude the possibility that mitomycin treatment inactivated precultured cells as stimulators, or that mitomycin-treated precultured stimulating cells might have an inhibitory effect on responding cells. Two-way mixed cultures without mitomycin treatment gave virtually identical results. The same cells, A and B, gave control counts of 260 ± 13 and 192 ± 18, respectively, when cultured alone. The mixture of A and B gave counts of 186 ± 34. Thus, both cells, which had been shown to respond to mitomycin-treated fresh lymphocytes, did not respond when reacted against each other. Two-way cultures of one fresh and one precultured cell always gave the expected stimulation. In addition, a four-fold dose of mitomycin-treated precultured cells, when added to a mixed culture of two fresh cells, had no inhibitory effect on lymphocyte transformation. Similar results were obtained in five additional identical experiments.

Table 2. Precultured and fresh lymphocytes in one-way mixed lymphocyte cultures. Cells A and B were precultured for 7 days; C and D were fresh. HL-A types (in parentheses), determined when the cells were fresh or after preculturing, were the same. The loss of ability to stimulate in MLC with retained ability to respond is apparent in cells A and B. Stimulating cells (A_m, B_m, C_m, and D_m) were produced by treating cells A, B, C, and D with mitomycin C. Incorporation of [^{3}H]thymidine was the measure of stimulation.

Responding cells	Item	Stimulating cells			
		Precultured 7 days		Freshly prepared cells	
		A_m	B_m	C_m	D_m
A; (3, 10, W5, W14); precultured 7 days	Count/min ± S.E.	145 ± 28	124 ± 24	5199 ± 801	5087 ± 679
	Stimulation ratio	1.00	0.86	35.69	34.92
B; (W24, W27); precultured 7 days	Count/min ± S.E.	105 ± 19	99 ± 2	5369 ± 244	6115 ± 636
	Stimulation ratio	1.06	1.00	53.88	61.35
C; (1, W32, 13); fresh	Count/min ± S.E.	1489 ± 227	1243 ± 88	1411 ± 143	9399 ± 567
	Stimulation ratio	1.06	0.88	1.00	6.66
D; (1, 10, W5, W16); fresh	Count/min ± S.E.	732 ± 27	489 ± 72	8410 ± 388	345 ± 88
	Stimulation ratio	2.12	1.42	24.36	1.00

The relation between time of preculturing and the ability of lymphocytes to stimulate or to respond was studied in 388 one-way MLC tests of allogeneic cells (Fig. 1). A progressive loss of stimulating activity of precultured cells with longer preculturing time can be seen, reaching a level of borderline significance after 1 week and becoming negative thereafter. In contrast, the ability of the same precultured cells to respond to fresh allogeneic cells shows no significant decrease for up to at least 10 days.

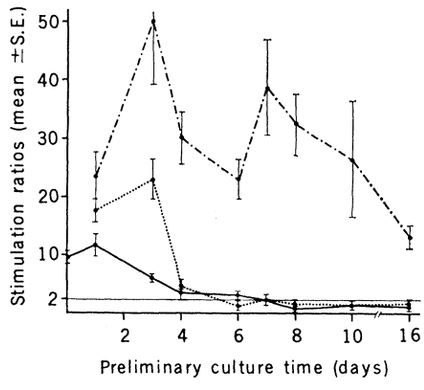

Fig. 1. Antigenicity and responsiveness of precultured lymphocytes as studied in one-way MLC. The thin horizontal line indicates the stimulation level (ratio of 2) which is generally considered to be significant. The values given are means ± S.E. of the stimulation ratios of individual experiments. Varying degrees of genetic disparity within the random test population result in variable stimulation ratios and standard errors. The loss of antigenicity with retained responsiveness can clearly be seen in precultured cells. ———, Fresh responding cells stimulated by precultured cells (174 combinations tested); . . . , precultured responding cells stimulated by cells precultured for the same number of days (115 combinations tested); – · – · –, precultured responding cells stimulated by fresh cells (99 combinations tested).

This clear separation of the stimulating and responding function of lymphocytes has many potential consequences. It may provide evidence for an MLC locus (*5*) product on the surface of lymphocytes, which is released or not sufficiently synthesized upon culturing. Blastogenic factors released into the culture medium in MLC (*6*) could be such products. Although HL-A typing could be performed without difficulty after preculturing, reduction of HL-A antigen on the cell membrane below threshold levels necessary for stimulation might occur. The turnover of membrane HL-A antigens on lymphocytes (*7*) may be decreased upon preculturing, resulting in loss of ability to stimulate. Structural alteration of microvilli (*8*) on lymphocytes upon culturing might render cells unable to stimulate, but might not affect allogeneic recognition and transformation. Interestingly, lymphoblast lines maintained in long-term cultures retain their ability to stimulate (*9*). Whether this is due to cell activation or induction of new antigenic sites by Epstein-Barr virus has not been determined.

Our results could also be explained by a differential loss or inactivation of a subpopulation of lymphocytes that is responsible for MLC stimulation. The cells shown as nonviable by trypan blue staining could possess stimulating antigens, whereas the remaining viable cells could lack them. Whether B (bone

marrow-derived) cells may be more capable of stimulating (*10*) and may be lost differentially remains to be determined. The decrease in the counts per minute of precultured cells when stimulated with PHA suggests some alteration of T (thymus-derived) cells during preculturing.

These findings may lead to a better understanding of the mechanisms of allogeneic cell recognition and stimulation. The common concept of lymphocyte responsiveness being a more active process than the passive stimulation function could prove incorrect.

Loss of immunogenicity upon culturing is important for organ transplantation, as already suggested by Summerlin (*2*). If the loss of immunogenicity of cultured skin is attributed to an effect on passenger lymphocytes, our findings could account for the transplantability of cultured skin. The extent to which this phenomenon can be utilized in the transplantation of bone marrow and other organs is still unknown.

In summary, a basic function of lymphocytes, namely their ability to stimulate allogeneic lymphocytes, has been shown to be independent of another basic function—their ability to respond to allogeneic lymphocytes and to PHA mitogen.

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Liquid Chromatographic Assay of Warfarin: Similarity of Warfarin Half-Lives in Human Subjects

Abstract. *A high pressure liquid chromatographic assay was developed to measure warfarin concentrations in biological fluids. Twelve healthy, unrelated volunteers received a single oral dose of warfarin (0.75 mg per kilogram of body weight). The mean plasma warfarin half-life was 36.3 ± 3.5 hours by liquid chromatography but 55.9 ± 8.4 hours by a currently used fluorimetric assay that fails to separate warfarin from its metabolites. Interindividual variation was greater and each half-life longer by the fluorimetric than by the chromatographic procedure. Warfarin shows less interindividual variation than that observed for other drugs primarily metabolized by hepatic microsomal mixed function oxidases. Advantages of specificity, rapidity, sensitivity, accuracy, and simplicity recommend liquid chromatography in the development of other drug assays.*

The oral anticoagulant warfarin is commonly used therapeutically in thrombophlebitis, pulmonary embolism, and myocardial infarction; its measurement in human blood facilitated many investigations on drug interactions and induction of hepatic microsomal mixed function oxidases (*1*). The genetic mechanism for the development of resistance to its use as a rodenticide has been reported (*2*), as have two human pedigrees with resistance to the hypoprothrombinopenic effects of warfarin (*3*). Elucidation of these phenomena depended on measurement of warfarin in biological fluid by a spectrophotometric (*4*) or fluorimetric assay (*5*). More recently, Lewis *et al.* (*6*) introduced a method for warfarin determinations based on thin-layer chromatography which separated warfarin from its metabolites. Lewis *et al.* (*6*) also showed that, when assayed for warfarin, blood specimens drawn serially after a single oral dose of warfarin in human subjects gave different clearance rates by the spectrophotometric (*4*) and the fluorimetric (*5*) methods. However, Welling *et al.* (*7*), using a smaller oral dose of warfarin, failed to confirm the observations of Lewis *et al.* (*6*). Lewis (*8*) refuted Welling *et al.* (*7*), and Wagner (*9*) replied. Hewick and McEwen (*10*) confirmed Lewis's work and extended his observations by reporting different plasma half-lives and anticoagulant efficacies for the enantiomers of warfarin in man.

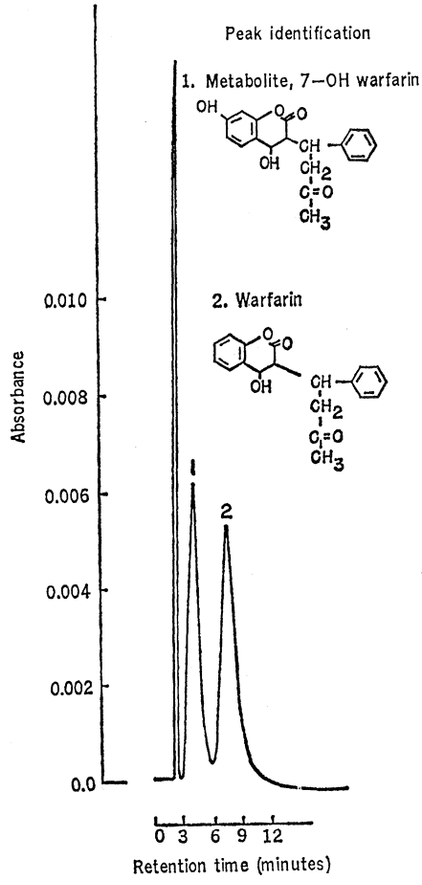

Fig. 1. Separation of warfarin and 7-hydroxywarfarin by high pressure liquid chromatography.

Increasing interest in warfarin pharmacokinetics and in measuring concentrations of other drugs prompts us to describe a simple assay of warfarin by high pressure liquid chromatography (LC) and to suggest the suitability of LC for quantitative estimation of other drugs. Advantages of LC over gas-liquid chromatography include elimination of an occasionally difficult derivatization step and also of volatilization which may fragment certain thermally unstable compounds.

The DuPont model 830 high pressure liquid chromatograph was used. A column (1 m by 2.1 mm) was packed with octadecylsilane (Permaphase). The mobile phase consisted of 10 percent dioxane in 90 percent H_2O at *p*H 4. The flow rate was 0.75 ml/min. The