Title
Evidence Concerning the Origin of Liver Macrophages

Authors
Kelly, Lola S
Dobson, Ernest L

Publication Date
2023-09-06

EVIDENCE CONCERNING THE ORIGIN OF LIVER MACROPHAGES

Lola S. Kelly and Ernest L. Dobson
From Donner Laboratory, Lawrence Radiation Laboratory,
University of California
Berkeley, California, 94720 U.S.A.

DISCLAIMER

SUMMARY

Chemical stimulation with endotoxin, estradiol or glucan has been used in conjunction with irradiation to modify various dynamic aspects of the RE system. Specific liver and spleen irradiation has been found to be as effective as whole body irradiation in suppressing the normal increase in RES activity and cell proliferation in the estradiol stimulated mice. In contrast, irradiated animals were found to continue to respond to endotoxin stimulation. It has been concluded, therefore, that endotoxin stimulates largely by activation of existing cells while estradiol stimulates largely by cell proliferation. Though these phagocytic cells in the liver may have had ancestral precursors from the bone marrow, we conclude from cell proliferation studies in stimulated animals that cell division of existing littoral cells is the predominant source of the increased number of hepatic macrophages.

INTRODUCTION

The phagocytic function of the reticuloendothelial system (RES) is stimulated by estrogens (Heller et al., 1957; Bilbey and Nicol, 1958) and by bacterial endotoxins (Benacerraf and Sebestyen, 1957; Howard, 1959; Arredondo and Kampschmidt, 1963; Kampschmidt et al., 1965). We have shown (Kelly et al., 1960, 1962) that the functional stimulation by estradiol is accompanied by marked proliferation of RE cells in the liver, and Howard (1959) has presented evidence that bacterial endotoxins cause an activation of existing cells with little if any increase in cell number.

Recently, Kinsky et al. (1969) have proposed that normal Kupffer cells are not a separate self replicating population of phagocytes but rather can be replaced and augmented by recruitment from a precursor in the bone marrow. The data to be presented below would indicate that this can only be a minor source of liver phagocytes. These studies involve the kinetics of cellular proliferation and phagocytic activity in irradiated and control animals.

METHODS

Irradiation

Irradiation was administered by the intravenous injection of particulate chromic phosphate containing ^{32}P. Suitably prepared (Dobson et al., 1966), this particulate material localizes rapidly and specifically in the liver and spleen. Only traces are found elsewhere in the body.

In unpublished studies, it was found that the radiation dose from the intravenous injection of one μ Ci of $Cr^{32}PO_4$ per gram of body weight resulted in no weight loss in mice. Three times this dose resulted only in a 10% loss in weight and no deaths during the nine month period of observation. Thus, this method provides a means of irradiating only those organs responsible for the clearance of colloidal carbon from the blood and avoids the debilitating effect of whole body irradiation.

Therefore, one microcurie of $Cr^{32}PO_4$ per gram of body weight was injected into adult male Swiss mice (25-30 gms). The mean concentration found in the liver was 17μCi/gm. When radiative losses from the surface and thin edges are considered (Jones et al., 1944), this concentration delivers an initial dose rate to the liver of 470 Rad per day, an integrated dose of 1360 Rad over a 3 day period and 3800 Rad over a 10 day period.

The splenic uptake of $CrPO_4$ varies considerably, from 1½ to 5 or 6 per cent. Spleen size relative to liver size is also quite variable. Therefore the dose received by the spleen cannot be determined with accuracy but was estimated at somewhat less than half that received by the liver.

Measurement of RES Activity

Phagocytic function was determined essentially by the method used by Benacerraf et al. (1954). Six mg per mouse of colloidal carbon(1) were injected intravenously. Blood was collected from the tip of the tail at appropriate time intervals.

The concentration of the carbon was measured spectrophotometrically and plotted on semilog paper as a function of time. The fractional disappearance rate constant, d, defined by

$$C = C_o e^{-kt}$$

has been used as a quantitative measure of phagocytic activity of the RE system [2].

RES Stimulation

RE system stimulation was produced by subcutaneous injection of 1.0 mg estradiol (Mann Research Laboratories, General Biochemicals) in 0.2 ml sesame oil or by intravenous injection of 0.1 mg E. coli endotoxin (Difco lipopolysaccharide 026:B6) in 0.1 ml isotonic saline. (The subcutaneous injection of sesame oil was found to have no effect on carbon clearance.)

In one experiment, 0.5 mg of glucan (Fleischmann Laboratories) in isotonic saline was injected intravenously. The glucan was prepared for injection by grinding a suspension in a Potter-Elvehjem homogenizer for 20 minutes.

Determination of Cell Populations

Relative numbers of littoral and parenchymal cell nuclei were determined from liver sections cut 6μ thick and stained with hematoxylin and eosin. (The term littoral cell rather than reticuloendothelial cell has been used after Abercrombie and Harkness (1951) because some uncertainty has existed as to whether or not all the sinusoidal lining cells are capable of phagocytosis and thus whether or not they belong to the reticuloendothelial system.) An oil immersion objective was

used to examine randomly chosen fields from the central portion of each liver. Five animals were used, and 300 cells were counted per liver.

Acid Phosphatase

Staining for acid phosphatase was performed according to the procedure recommended by Novikoff (1960).

Thymidine Incorporation

Tritiated thymidine was used as a tracer to assess the extent of DNA synthesis. In one experiment, one μCi of ^{3}H thymidine (Schwarz BioResearch, Inc.) per gram of body weight was injected intraperitoneally into 8 week old male mice at one, two, three, four and seven days after stimulation by either estradiol or endotoxin and into untreated mice as controls. The mice were sacrificed one hour after the ^{3}H thymidine was given. Livers were fixed, embedded in paraffin and sectioned at six microns. They were covered with autoradiographic stripping film (Kodak AR-10), exposed for 3 weeks, developed and stained with hematoxylin and eosin. The percentage of labeled littoral cells was determined by scoring random fields at a magnification of 900 X, avoiding areas containing portal triads or central veins. Approximately 1000 cells were examined for each liver, and at least four livers were averaged per point.

In other experiments, the mice were sacrificed at varying times after thymidine injection, and the livers prepared for autoradiography as described above.

RESULTS

The Effect of RES Irradiation on Subsequent RES Stimulation

The time sequence. Fig. 1 shows the effect of estradiol on the carbon disappearance rate constant, k, in irradiated and nonirradiated mice compared as a function of time. There was a very marked and progressive increase in the value of k with time in the unirradiated animals. This increase was very greatly suppressed by the specific $Cr^{32}PO_4$ liver and spleen irradiation, which was 2800 Rad at the time estradiol was given at seven days and an additional 1200 Rad during the four days of stimulation. These irradiated estradiol treated animals did, however, show a slight stimulation when compared with the irradiated control mice. It is interesting to note, too, that specific liver and spleen irradiation resulted in only a slight depression in the carbon clearance rate when compared to normal controls, attesting once again to the relative radioresistance of the phagocytic process itself.

The comparison of endotoxin and estradiol stimulation. The effect of chromic phosphate irradiation and of estradiol and endotoxin on body weight, liver weight and spleen weight are presented in Table 1. This dose of radiation, limited to the liver and spleen, produced only a slight retardation of the normal gain in body weight. However, spleen weights were markedly reduced by the irradiation as expected, since the spleen which is radiosensitive takes up chromic phosphate and receives a radiation dose roughly comparable to that of the liver.

The phagocytic function, expressed as a disappearance rate constant, of irradiated and nonirradiated mice stimulated with estradiol or endotoxin is summarized in Table 2. In all experiments, the rate of carbon disappearance was measured three days after giving the estradiol or endotoxin. The stimulation with estradiol was generally greater than the stimulation with endotoxin in the dosages administered.

In experiments 1 and 2, the chromic phosphate was given seven days before the estradiol or endotoxin, resulting in a total exposure of the liver to 3800 Rad at the time the carbon clearances were measured at 10 days. In experiment 3, the chromic phosphate was given one hour before the estradiol and endotoxin. In this case, the radiation intensity at the time of stimulation was somewhat higher, but the total dose delivered at the time the carbon clearances were measured was only 1360 Rad.

The degree of stimulation in the irradiated mice was compared to the degree of stimulation in control (unirradiated) mice. This comparison was accomplished by dividing the carbon clearance rate constant of the stimulated animals by the carbon clearance rate constant of the unstimulated animals. These ratios are listed in Table 2. The ratio in irradiated animals was then divided by the ratio in unirradiated animals. These calculations, presented in Table 3, show that the irradiation of the liver with chromic phosphate depressed the estradiol effect to half but had no effect on the endotoxin stimulation.

The effects of estradiol and of endotoxin on liver cell

populations in irradiated and nonirradiated livers are presented in Table 4. It is apparent that estradiol produced a very marked increase in littoral cell number and that this increase was inhibited by irradiation. Endotoxin, on the other hand, produced only a very small rise in the littoral cell population. This increase also was prevented by the irradiation.

It has been suggested that the histologically demonstrable acid phosphatase content of phagocytic cells is in some way associated with their phagocytic activity (Howard, 1959; Thorbecke et al., 1961; Cohn and Benson, 1965). A marked increase in acid phosphatase staining in littoral cells was observed at one and three days after the administration of endotoxin, confirming the observations of Howard (1959). A very similar increase in acid phosphatase staining was also produced by estradiol. Specific liver irradiation (3800 Rad in 10 days) delivered by particulate $Cr^{32}PO_4$ produced no observable alteration in the acid phosphatase response to endotoxin.

Whole Body Irradiation

If the bone marrow were a significant source of phagocytes following stimulation, then whole body irradiation should result in a much greater suppression than specific liver and spleen irradiation. An experiment was therefore performed in which mice were irradiated acutely with 600 whole body X irradiation. Subsequently, on the day of irradiation, they were injected with either estradiol or endotoxin, and carbon clearances were measured three days later. The carbon clearance data are presented in Table 5. The degree of stimulation in the

irradiated mice as compared to the degree of stimulation in the control mice was calculated in the same manner as for the specific liver and spleen irradiated mice discussed previously. The results of this calculation are presented in Table 3 in order that a comparison may be made between the effects of whole body irradiation and the specific liver and spleen irradiation. It is evident from this table that for estradiol there was no significant difference in the results obtained from the two irradiation schemes. Thus it appears the bone marrow is not a significant source of phagocytic cells following this mode of stimulation.

In the case of endotoxin, however, a depression was observed in the whole body irradiated animals as compared to the specific liver and spleen irradiated animals. The data here are much more variable than is the case with estradiol so that it cannot be established that this represents a real depression. In any event, considerable stimulation is evident in spite of heavy irradiation when endotoxin was used as the stimulant. This heavy irradiation must preclude all new cell formation, and the very marked increase in activity can be explained only on the basis of cell activation.

Cell Division and Population Changes

Fig. 2 shows one hour thymidine labeling of littoral cells as a function of time after administration of estradiol or endotoxin to unirradiated animals. Estradiol appeared to stimulate DNA synthesis to a slightly greater extent than did the endotoxin, though the stimulation by endotoxin was quite marked.

It should be noted that these data are derived from mice sacrificed only one hour after thymidine administration. Therefore, the labeled cells represent a population which was resident in the liver at that time. This does not preclude the possibility that some of their precursors might have originated elsewhere (Boak et al., 1968; Kinsky et al., 1969).

Cell population changes as a function of time after stimulation by estradiol or endotoxin in these same animals are shown in Fig. 3. Because the parenchymal cells showed essentially no thymidine incorporation and therefore presumably no population change, they have been used as a normalizing factor. At 4 days after estradiol administration, the littoral cell population had approximately doubled. Endotoxin treatment, on the other hand, resulted in a very much smaller increase. The large amount of thymidine labeling in endotoxin treated animals (Fig. 2), together with the relatively small increase in cell number (Fig. 3), suggests that either the endotoxin stimulated cells did not divide or that there was some cell death.

Littoral Cell Kinetics

An experiment was performed to examine the behavior of thymidine labeled clls which were produced as a result of estradiol stimulation. Mice were injected with 1 mg of estradiol and given thymidine three days later. Some of the animals were sacrificed at one hour and other groups on various days up to two weeks after thymidine administration. The data are presented in Fig. 4.

One hour after thymidine administration 10 per cent of the littoral cells in the estradiol stimulated animals were labeled. Because the time interval is so short it must be concluded that these cells were resident in the liver and in the process of DNA synthesis at the time of thymidine administration. Mice sacrificed one day after thymidine injection showed 14 per cent of their littoral cells labeled. Theoretically, if all of the cells which were labeled at one hour had divided, the percentage of labeled cells should have nearly doubled. Furthermore, if there had been in addition an appreciable influx of labeled cells from the marrow or elsewhere, the percentage of labeled cells in the liver should have been even higher than this. In fact, the observed one day value was only 40 per cent above the one hour value.

The labeled cell population in these estradiol treated animals decreased surprisingly rapidly with a half time of roughly 4 to 5 days. The controls on the other hand remained relatively constant at 1 to 2 per cent labeled cells over the 11 day period of observation. The rapid decline in labeled cells in the estradiol treated animals after day 4 was accompanied by a return toward normal of the total littoral cell population as shown in Fig. 3.

Mean grain counts dropped to roughly half, as expected for dividing cells, between one hour and one day and then did not decrease much further during the remaining days of observation. While grain counts determined with tissue sections

are notoriously fraught with problems, the data indicate that, on the average, the labeled cells divided only once and therefore the disappearance of labeled cells could not be accounted for on the basis of dilution of the label.

In order to further investigate the possibility and the magnitude for an influx of newly formed cells into the liver in response to stimulation, an experiment was done using glucan as the RES stimulant (Riggi and DiLuzio, 1961). In contrast to the experiment described above, thymidine was administered one day before the intravenous injection of 0.5 mg of glucan. The glucan treated mice and their appropriate controls were sacrificed daily thereafter. In addition, another group was stimulated with glucan three days prior to the thymidine. These animals were sacrificed at one hour in order to assess the percentage of cells resident in the liver which were synthesizing DNA at that time. The results are presented in Table 6.

The animals which were given glucan subsequent to thymidine labeling showed no difference from the unstimulated controls except for a very slight elevation on the second day after glucan stimulation. In contrast when thymidine was given to animals which had been injected with glucan three days previously, three times as many littoral cells had incorporated thymidine in one hour. The labeling response to stimulation of this group was similar to though lower than that seen in Fig. 2 and Fig. 3 in which estradiol and endotoxin were used as stimulants.

DISCUSSION

Two main points emerge from the experiments reported here. An increase in the functional activity of the RE system in its ability to remove colloids from the blood may result from an activation of pre-existing cells, or it may result from an increase in littoral cell number in the liver. The second point is concerned with the origin of the additional littoral cells. Do these arise mainly from the division of cells already resident in the liver? Or are they recruited from some extra-hepatic source as suggested by Kinsky et al. (1969)?

With the exception of one recent report (Saba and DiLuzio, 1969) which has shown a marked inhibition of the RES phagocytic activity by irradiation, the phagocytic function of the RES has been almost universally found to be radioresistant. In contrast to this radioresistance, it has been established that x-irradiation does prevent the stimulation of the RES by zymosan (Benacerraf et al., 1969; Wooles et al., 1962; Fred et al., 1969). This stimulation is associated with proliferation of the RE cells of the liver (Kelly et al., 1960). And indeed, radiation, which is known to suppress cell division, was found to markedly inhibit stimulation of the RE system by estradiol but left unaffected the stimulation by endotoxin (Fig. 1, Tables 2, 3, 5). Therefore, it is suggested that there exists a qualitative difference in the mechanism by which these two agents act. As we have suggested previously (Kelly and Dobson, 1961), estradiol appears to stimulate phagocytic function primarily by proliferation of the RE elements.

Endotoxin, on the other hand, seems to produce its effect largely by cell activation.

While estradiol stimulation was reduced by liver irradiation, it is clear from Tables 2, 3 and 5 that there was nonetheless a significant stimulation in the irradiated animals which suggested that estradiol also activated the cells. This was confirmed not only by the increased liver acid phosphatase in estradiol treated animals but also by the observation, Fig. 1, that phagocytic function was somewhat increased in mice one day after estradiol administration. This interval is too short for an increase in cell number by cell division (see Figs. 2 and 3).

On the other hand, though endotoxin stimulation in the experiments reported here produced an appreciable increase in DNA synthesis (Fig. 2), the end result was only a slight increase in littoral cell number (Fig. 3). This latter observation is in agreement with data reported by Howard (1959). The marked increase in the DNA synthetic rate without a concomitant increase in cell population in unirradiated mice suggested either failure of the cells to divide or cell death, presumably from the endotoxin. Forbes (1965) also has suggested that macrophages are damaged by endotoxin. His data too showed that thymidine incorporation increased markedly in peritoneal macrophage populations after endotoxin administration. Silverman (1965) has also demonstrated a marked sensitivity of irradiated macrophages to killing by bacterial endotoxins.

In a recent paper, Kinsky et al. (1969) have shown convinc-

ingly that macrophage populations in the liver can be augmented by precursor cells from the bone marrow. The experiments presented above indicate to us that under our experimental conditions this can only be a minor source of the increased littoral cell number.

The increase observed in the estradiol treated animals can be accounted for with ease by division of littoral cells already resident in the liver at the time of stimulation. In a previous paper we have demonstrated that cells which are in the process of DNA synthesis in the liver belong to a mature population of cells which is capable of phagocytosis (Kelly et al., 1962). This does not preclude that some of these cells may have had ancestors from the bone marrow. The timing observed in the increase in DNA synthesis in littoral cells after stimulation is very similar to the timing observed by Edwards and Koch (1964) for mouse livers regenerating after partial hepatectomy.

It appears to us that the littoral cell population in the liver has very different dynamics from the macrophage population in the peritoneal cavity. In studies of the peritoneal macrophages, Volkman and Gowens (1965) and Chen and Schooley (1970) have shown a very rapid influx of about 30% of newly formed cells from the marrow as a result of stimulation. Our attempt to find a similar phenomenon in the liver was unsuccessful. However, it is important to remember that the mouse liver contains approximately 3×10^8 macrophages while, at any

one time, there are only 6×10^6 macrophages in the peritoneal cavity. If the same number of cells, namely 2×10^6, migrated to the liver from the marrow in a day, it would be such a small fraction of the total population present as to escape detection.

The 1-2% littoral cell labeling by tritiated thymidine which we have continually observed in unstimulated animals suggests a long lived cell. This cell longevity is confirmed by experiments reported by Samis et al. (1966) who gave repeated injections of ^3H thymidine over a period of 42 days and found no more than 20% labeled littoral cells at the end of that long period of time.

In a previous study we showed that the value of k returned toward normal more rapidly following estradiol stimulation than one might expect (Kelly et al., 1960). Ware and Nicol (1960) have made a similar observation. Fig. 4 shows that labeled cells produced in response to estradiol disappear quite rapidly as well. In fact, the total cell population which had increased in response to estradiol also returned rapidly toward normal (Fig. 3). It is not known whether these cells die or whether they move elsewhere and augment other macrophage populations in the body. Roser (1968) has shown that when macrophages are injected intravenously they are capable of settling in various tissues and continue to function as macrophages. Thus, some mobility is within the realm of possibility.

Footnotes

1. Gunther Wagner, Hanover, German, Suspension no. C11/1431a, prepared as described by Parker and Finney (1960).
2. The approximation of the disappearance curve to an exponential function and the relationship of the disappearance constant to the "phagocytic index" is discussed by Dobson *et al.* (1967).

Figure Legends

Fig. 1. The effect of specific liver-spleen irradiation on estradiol stimulation of the RES as measured by the increase of the carbon disappearance rate constant. Values plotted are k x 100, plus or minus one standard error of the means, as a function of time.

Fig. 2. The ^{3}H thymidine labeling of the littoral cells in autoradiographs of the liver as a function of time after administration of estradiol or endotoxin. The ^{3}H thymidine was administered 1 hour before sacrifice. Bars indicate plus or minus one standard error of the mean. The point at 0 time represents the labeling in normal, untreated mice.

Fig. 3. Liver cell population changes as a function of time after estradiol or endotoxin stimulation. Bars indicate plus or minus one standard error of the mean. The point at 0 time represents the cell population ratio in normal, untreated mice.

Fig. 4. Time course of littoral cell labelling following a single injection of thymidine delivered three days after estradiol administration. The controls received only the single injection of thymidine at the time indicated by the arrow.

Table 1

Body Weight and Organ Weight as Percent of Initial Body Weight

	Body Wt. Percent*	Liver Wt. Percent*	Spleen Wt. Percent*
Experiment 1 **			
Unirradiated Controls	110 ± 1	6.3 ± 0.2	0.42 ± 0.22
" Estradiol	117 ± 1	8.0 ± 0.2	0.63 ± 0.07
" Endotoxin	100 ± 1	6.6 ± 0.2	0.84 ± 0.03
$Cr^{32}PO_4$ - Irradiated	104 ± 1	5.6 ± 0.2	0.26 ± 0.01
" Estradiol	109 ± 2	6.6 ± 0.3	0.36 ± 0.03
" Endotoxin	98 ± 1	6.2 ± 0.2	0.37 ± 0.03
Experiment 2 **			
Unirradiated Controls	108 ± 2	5.3 ± 0.2	0.39 ± 0.02
" Estradiol	111 ± 2	6.2 ± 0.2	0.49 ± 0.03
" Endotoxin	101 ± 1	5.9 ± 0.2	0.77 ± 0.03
$Cr^{32}PO_4$ - Irradiated	105 ± 1	5.4 ± 0.1	0.25 ± 0.01
" Estradiol	107 ± 2	5.4 ± 0.2	0.27 ± 0.01
" Endotoxin	98 ± 1	6.0 ± 0.2	0.40 ± 0.02
Experiment 3 **			
Unirradiated Controls	106 ± 1	6.2 ± 0.1	0.67 ± 0.05
" Estradiol	108 ± 2	6.9 ± 0.2	0.89 ± 0.06
" Endotoxin	100 ± 1	6.4 ± 0.3	1.00 ± 0.04
$Cr^{32}PO_4$ - Irradiated	102 ± 1	5.5 ± 0.2	0.43 ± 0.03
" Estradiol	108 ± 1	6.6 ± 0.2	0.54 ± 0.05
" Endotoxin	98 ± 1	6.6 ± 0.2	0.70 ± 0.05

* Ten animals were used per observation

** The irradiation of experiments 1 and 2 was delivered over a ten day period. In experiment 3, the period was three days. See text.

Table 2

Carbon Disappearance from the Blood of Mice*

	Unirradiated Mice		$Cr^{32}PO_4$ Irradiated Mice	
	Percent per Min. k x 100	Ratio to Control	Percent per Min. k x 100	Ratio to Control
Experiment 1				
Control	2.5 ± 0.01	——	1.8 ± 0.2	——
Estradiol	11.1 ± 1.3	4.43	4.1 ± 0.4	2.21
Endotoxin	6.1 ± 0.6	2.43	5.1 ± 0.3	2.75
Experiment 2				
Control	1.9 ± 0.2	——	1.7 ± 0.1	——
Estradiol	7.6 ± 1.0	3.94	3.0 ± 0.3	1.78
Endotoxin	6.4 ± 0.6	3.34	4.9 ± 0.3	2.92
Experiment 3				
Control	2.7 ± 0.2	——	2.4 ± 0.3	——
Estradiol	13.6 ± 2.2	5.00	7.2 ± 0.8	3.05
Endotoxin	7.6 ± 0.7	2.80	6.6 ± 0.9	2.76

* Each value is the mean of ten mice together with the standard error of the mean.

Table 3

The Efficiency of RES Stimulation in Irradiated Mice

	Liver–Spleen Irradiation			Whole Body Irradiation
Stimulant	Expt. 1	Expt. 2	Expt. 3	
	The calculations*are from Table 2.			The calculations*are from Table 5
Estradiol	0.50	0.45	0.61	0.61
Endotoxin	1.13	0.87	0.98	0.77

$$\frac{\text{*Stimulant effect in irradiated mice}}{\text{Stimulant effect in control mice}} = \frac{k_{(\text{stimulated + irradiated})}}{k_{(\text{irradiated})}} \div \frac{k_{(\text{stimulated})}}{k_{(\text{control})}}$$

Table 4

Liver Cell Populations* Four Days Post Endotoxin or Estradiol

	Unirradiated Controls	Estradiol Only	Endotoxin Only	$Cr^{32}PO_4$ Only	$Cr^{32}PO_4$ Estradiol	$Cr^{32}PO_4$ Endotoxin
Littoral cells/100 parenchymal cells	76	172	96	74	77	74
Std. error of the mean	± 2	± 11	± 3	± 8	± 4	± 3

* Each group was comprised of 5 mice.

Table 5

Carbon Disappearance from the Blood of Mice*

	Unirradiated Mice		Whole Body Irradiated Mice	
	Percent per Min. k x 100	Ratio to Control	Percent per Min. k x 100	Ratio to Control
Control	2.1 ± 0.1	___	2.0 ± 0.2	___
Estradiol	5.6 ± 0.6	2.69	3.3 ± 0.5	1.65
Endotoxin	8.1 ± 1.1	3.86	6.0 ± 1.2	2.98

* Each value is the mean of ten mice together with the standard error of the mean. 600R whole body x-irradiation, estradiol or endotoxin injected on the day of irradiation, and carbon clearances measured at 3 days.

Table 6

The Effect of Glucan Stimulation Before or After Thymidine: Per Cent Labeled Littoral Cells

Unstimulated Controls

Time after thymidine	1 hour	1 day	2 days	3 days	4 days
	2.25 1.71 2.02 } 1.99	2.89 0.63 1.01 } 1.51	1.93 1.50 3.33 } 2.25	1.22 1.43 1.32 } 1.32	0.98 2.02 2.04 } 1.68

Glucan Stimulated After Thymidine Administration

Time after thymidine	1 hour	1 day	2 days	3 days	4 days
Time after glucan		0 day	1 day	2 days	3 days
			1.80 1.69 3.00 } 2.16	2.91 4.37 1.73 } 3.00	1.36 1.82 1.80 } 1.66

Glucan Stimulated Before 1-Hour-Thymidine Administration

Time after thymidine				1 hour
Time after glucan	0 day	1 day	2 days	3 days
				3.16 9.72 5.76 } 6.21

References

Abercrombie, M. and Harkness, R. D. (1951). Proc. Roy. Soc., series B, 138, 544.

Arredondo, M. I. and Kampschmidt, R. F. (1963). Proc. Soc. Exptl. Biol. Med., 112, 78.

Benacerraf, B., Kivy-Rosenberg, E., Sebestyen, M.D. and Zweifach, B. W. (1959). J. Exper. Med., 110, 49.

Benacerraf, B., Halpern, B. M., Biozzi, G. and Benos, S. A. (1954). Brit. J. Exptl. Pathol., 35, 97.

Benacerraf, B. and Sebestyen, M. M. (1957). Fed. Proc., 16, 860.

Bilbey, D. L. J. and Nicol, T. (1958). Nature, 182, 674.

Boak, J. L., Christie, G. H., Ford, W. L. and Howard, J. G. (1968). Proc. Roy. Soc., series B., 169, 307.

Chen, M. G. and Schooley, J. C. (1970). Rad. Res., 41, 623.

Cohn, Z. A. and Benson, B. (1965). J. Exp. Med., 121, 153.

Dobson, E. L., Finkelstein, L. J., Finney, C. R. and Kelly, L. S. (1966). In "Radioactive Pharmaceuticals" (G. A. Andrews, R. M. Kniseley and H. N. Wagner, Jr., eds.), pp. 477. U. S. Atomic Energy Commission, Div. of Technical Information.

Dobson, E. L., Kelly, L. S. and Finney, C. R. (1967). In "Advances in Experimental Medicine and Biology" (N. R. DiLuzio and R. Paoletti, eds.), Vol. 1, pp. 63. Plenum Press, N.Y.

Edwards, J. L. and Koch, A. (1964). Lab. Invest., 13, 32.

Forbes, I. J. (1965). J. Immunol., 94, 37.

Fred, R. K., Dobson, E. L., Kelly, L. S. and Shore, M. L. (1970). RES, J. Reticuloendothelial Soc., 7, 453.

Heller, J. H., Meier, R. M., Zucker, R. and Mast, G. W. (1957). Endocrinol., 61, 235.

Howard, J. G. (1959). J. Pathol. and Bacteriol., 78, 465.

Jones, H. B., Wrobel, C. J. and Lyons, W. R. (1944). J. Clin. Invest., 23, 783.

Kampschmidt, R. F., Upchurch, H. F. and Park, A. (1965). RES, J. Reticuloendothelial Soc., 2, 256.

Kelly, L. S., Brown, B. A. and Dobson, E. L. (1962). Proc. Soc. Exptl. Biol. Med., 110, 555.

Kelly, L. S. and Dobson, E. L. (1961). Fed. Proc., 20, 265.

Kelly, L. S., Dobson, E. L., Finney, C. R. and Hirsch, J. D. (1960). Am. J. Physiol., 198, 1134.

Kinsky, R. G., Christie, G. H., Elson, J. and Howard, J. G. (1969). Brit. J. Exper. Pathol., 50, 438.

Novikoff, A. B. (1960). In "Developing Cell Systems and Their Control" (Rudnick, D. ed.), pp. 167, Ronald Press Co., N. Y.

Parker, H. G. and Finney, C. R. (1960). Am. J. Physiol., 198, 916.

Riggi, S. J. and Di Luzio, N. R. (1961). Am. J. Physiol., 200, 297.

Roser, B. (1968). J. Reticuloendothelial Soc., 5, 455.

Saba, T. M. and Di Luzio, N. R. (1969). Am. J. Physiol., 216, 910.

Samis, H. V., Jr., Falzone, J. A., Jr. and Wulff, V. J. (1966). Gerontologia, 12, 79.

Silverman, M. S. (1965). In "U. S. Armed Forces Epidemiological Board, Commission on Radiation and Infections, Annual Progress Report, 1965".

Thorbecke, G. J., Old, L. J., Benacceraf, B. and Clarke, D. A. (1961). J. Histochem. Cytochem., 9, 392.

Volkman, A. and Gowans, J. L. (1965). Brit. J. Exper. Pathol., 46, 62.

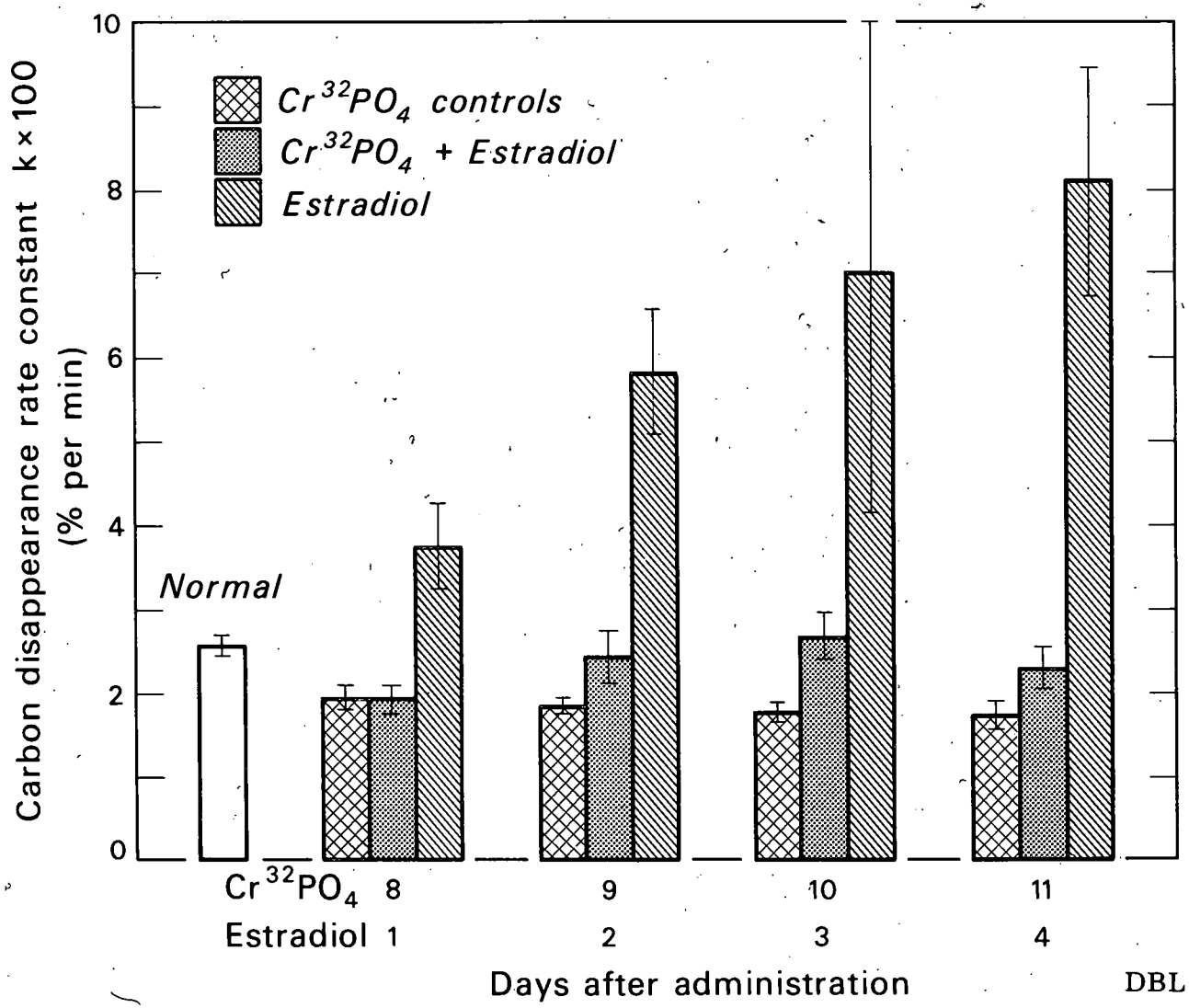
Ware, C. C. and Nicol, T. (1960). Nature, 186, 974.

Wooles, W. R., Elko, E. E. and Di Luzio, N. R. (1962). Rad. Res., 16, 546.

ACKNOWLEDGMENT

We wish to thank Mrs. Caroline R. Finney for her excellent assistance.

This work was supported by the U. S. Atomic Energy Commission.


Carbon disappearance rate constant k×100
(% per min)
10
8
6
4
2
0
$Cr^{32}PO_4$ controls
$Cr^{32}PO_4$ + Estradiol
Estradiol
Normal
$Cr^{32}PO_4$ 8 9 10 11
Estradiol 1 2 3 4
Days after administration

DBL 708 5867

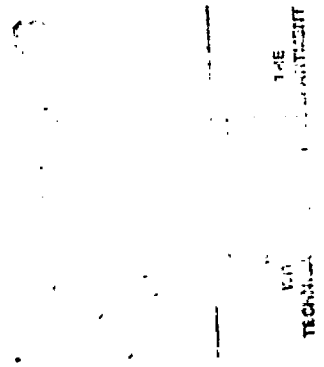
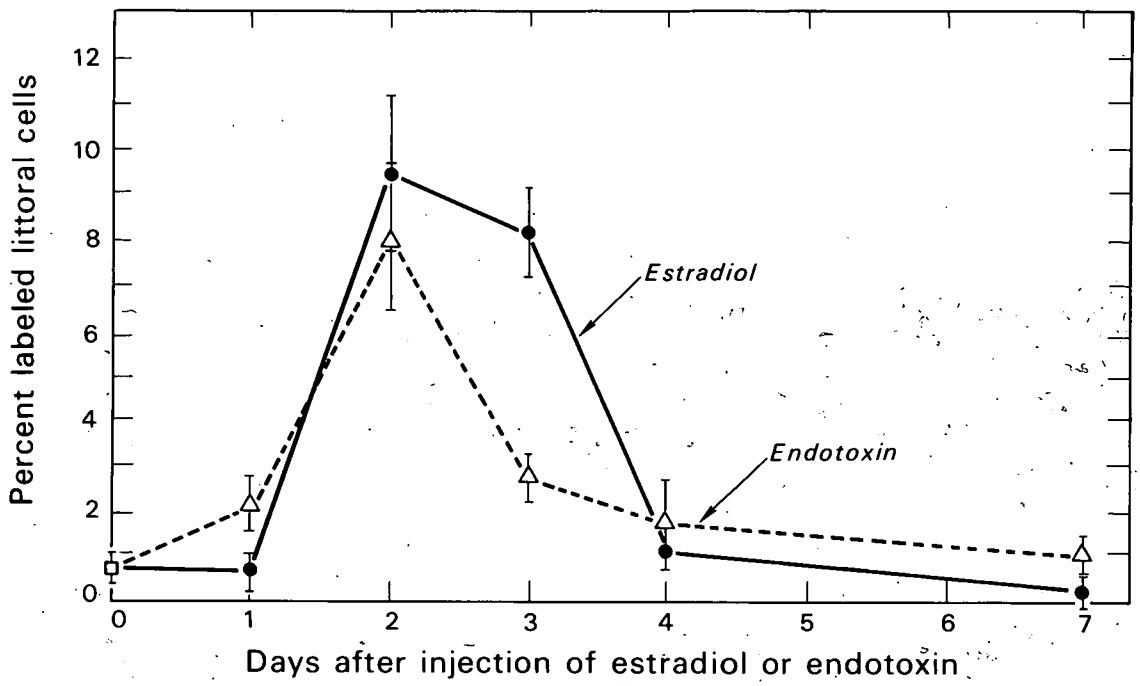


Fig 1

Kelly + Dobson

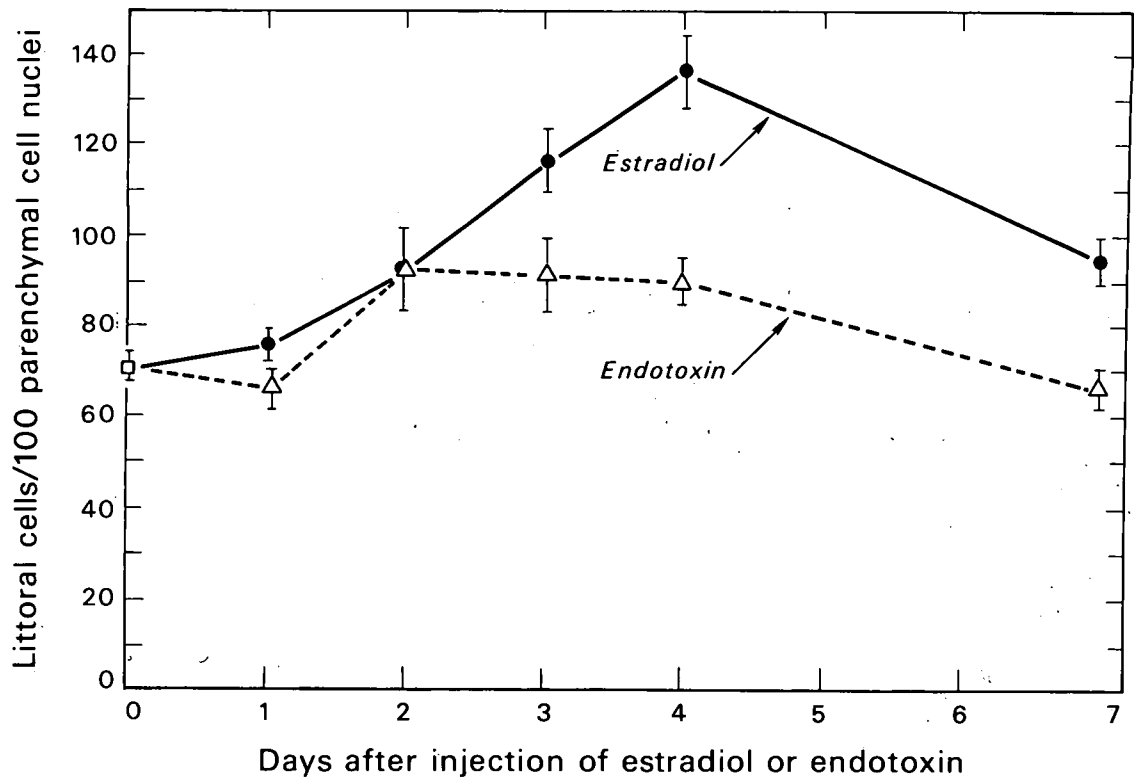


DBL 6911 5159

Top ↑

FIG. 2.

KELLY AND DOBSON

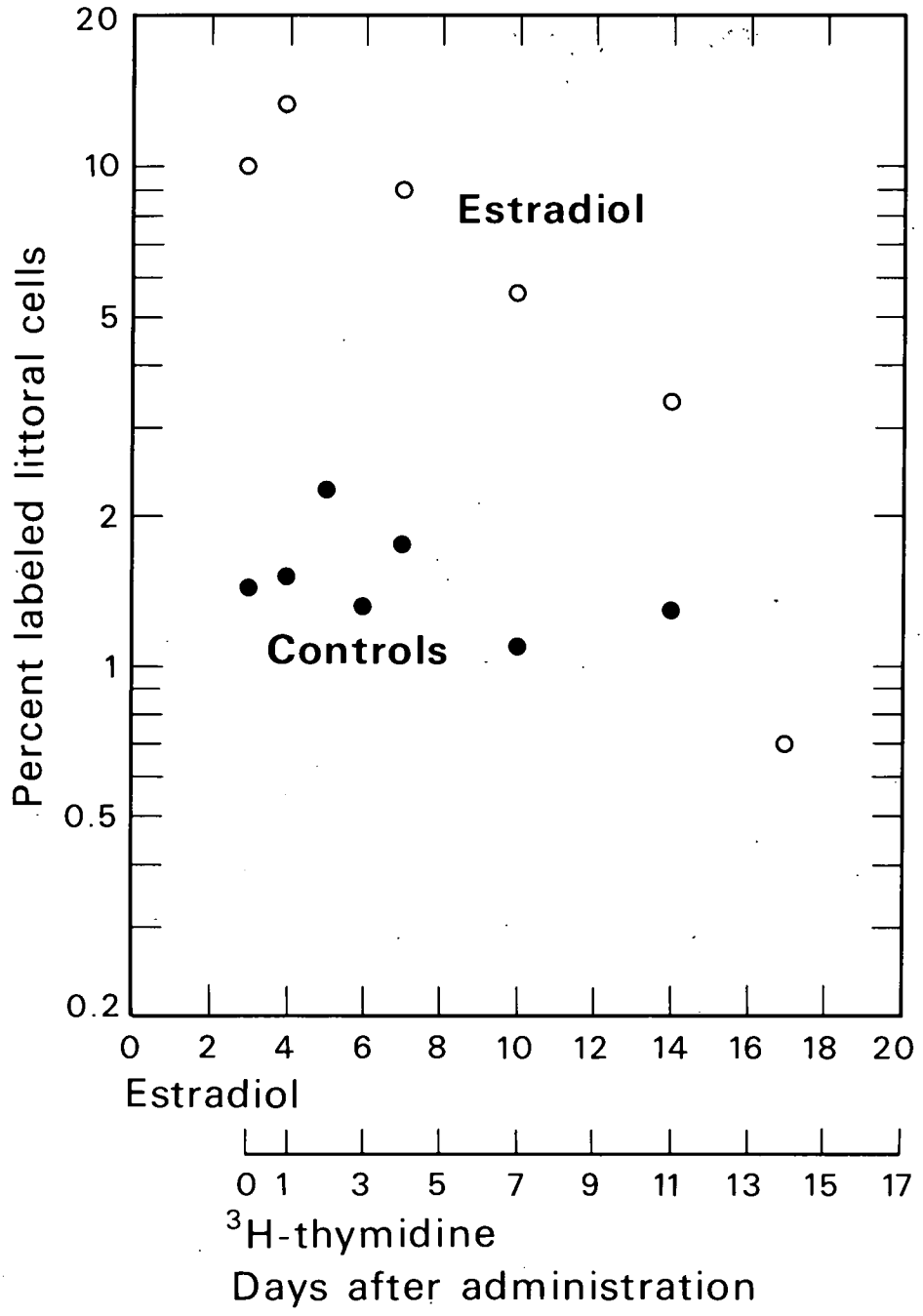


DBL 6911 5158

Top ↑

FIG. 3

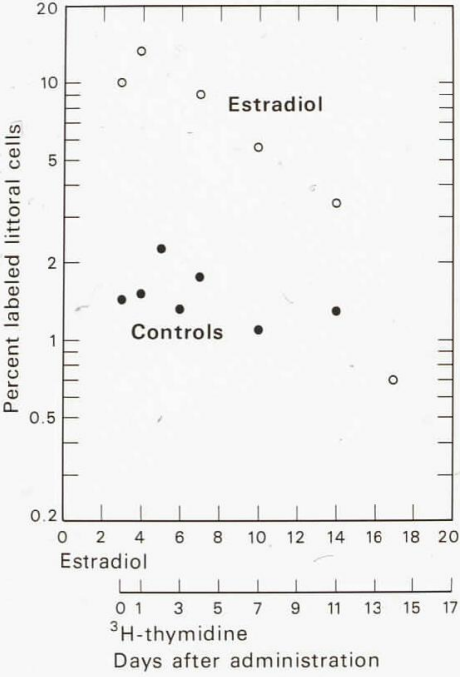
KELLY AND DOBSON


20
10
5
2
1
0.5
0.2
Percent labeled littoral cells
Estradiol
Controls
0 2 4 6 8 10 12 14 16 18 20
Estradiol
0 1 3 5 7 9 11 13 15 17
^{3}H-thymidine
Days after administration

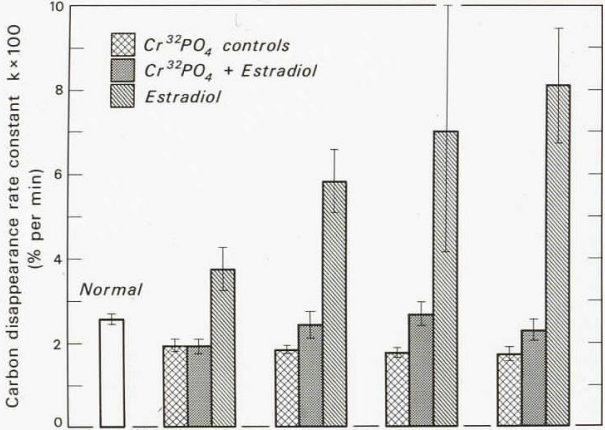
DBL 708 5866

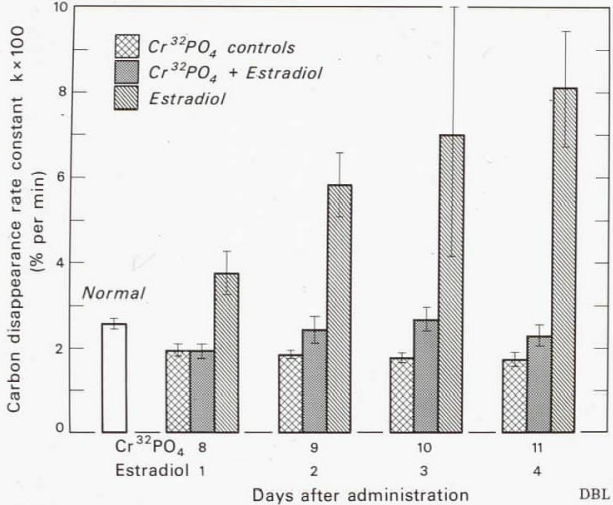
Fig 4

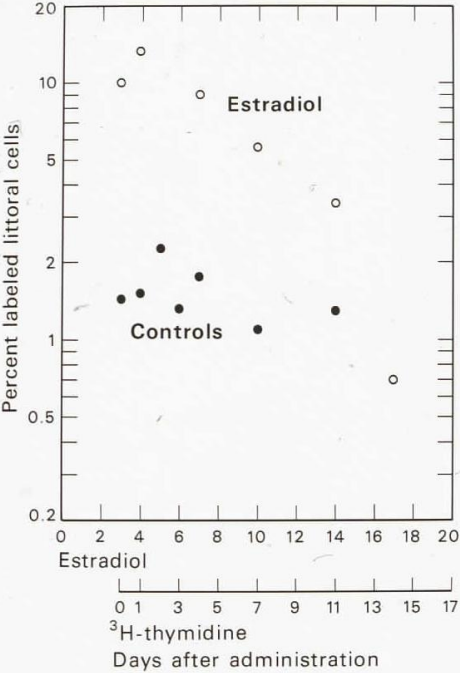
Kelly + Dobson


Percent labeled littoral cells
20
10
5
2
1
0.5
0.2
Estradiol
Controls
0 2 4 6 8 10 12 14 16 18 20
Estradiol
0 1 3 5 7 9 11 13 15 17
^{3}H-thymidine
Days after administration

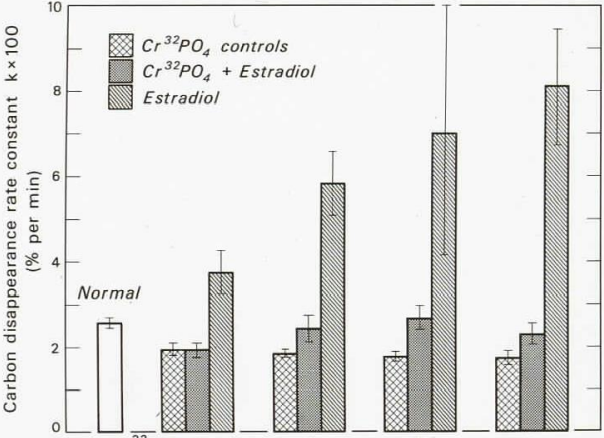
DBL 708 5866

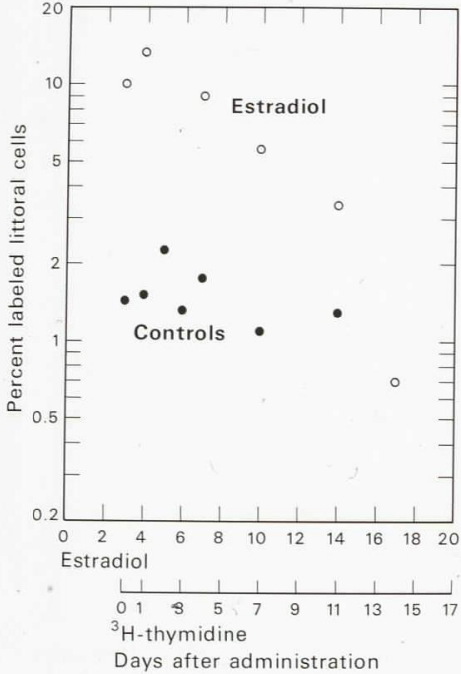

Carbon disappearance rate constant k×100 (% per min)
10
8
6
4
2
0
$Cr^{32}PO_4$ controls
$Cr^{32}PO_4$ + Estradiol
Estradiol
Normal
$Cr^{32}PO_4$ 8
Estradiol 1
9
2
10
3
11
4
Days after administration
DBL 708 5867


Carbon disappearance rate constant k×100
(% per min)
10
8
6
4
2
0
Cr 32PO4 controls
Cr 32PO4 + Estradiol
Estradiol
Normal
Cr 32PO4 8 9 10 11
Estradiol 1 2 3 4
Days after administration
DBL 708 5867


Percent labeled littoral cells
20
10
5
2
1
0.5
0.2
Estradiol
Controls
0 2 4 6 8 10 12 14 16 18 20
Estradiol
0 1 3 5 7 9 11 13 15 17
^{3}H-thymidine
Days after administration

DBL 708 5866


Carbon disappearance rate constant k×100
(% per min)
10
8
6
4
2
0
Cr 32PO4 controls
Cr 32PO4 + Estradiol
Estradiol
Normal
Cr 32PO4 8 9 10 11
Estradiol 1 2 3 4
Days after administration
DBL 708 5867


Percent labeled littoral cells
20
10
5
2
1
0.5
0.2
Estradiol
Controls
0 2 4 6 8 10 12 14 16 18 20
Estradiol
0 1 3 5 7 9 11 13 15 17
^{3}H-thymidine
Days after administration

DBL 708 5866