rectum, stomach, breast, and female genital) had degrees of multinucleation significantly higher than controls ($P < .02$). With the exception of a group containing six unrelated tumors, the mean degree of multinucleation in groups containing less than nine cases (urinary system, lymphomas, bronchogenic carcinomas, and digestive other than stomach, colon, and rectum), although comparatively as high as in all other groups, was not statistically significant. When the mean degree of multinucleation for all these small groups totaling 19 cases was calculated, it was found to be 4.25 ± 0.7 against 1.91 ± 0.3 for controls ($P < .01$).

When the percentage of smears falling into multinucleation groups rising by increments of 3 percentage points was calculated (Fig. 1), the majority of controls (83.9 percent) were found in the 0 to 2.99 percent multinucleation range and the majority of cancers (64.2 percent) above that range. There were always more cancers than controls when multinucleation exceeded 3 percent, and no control was found to have more than 6.25 percent multinucleated cells.

The mean high number of nuclei per cell per smear was 3.34 ± 0.15 for cases with tumors and 2.25 ± 0.08 for controls ($P < .01$).

Although significantly higher numbers of tracheobronchial ciliated multinucleated cells and a higher degree of multinucleation were found in patients with malignancies, and were not affected by sex, age, or smoking habit, it is impossible to foretell at present if this finding will remain consistent for all histological types of tumors when the series is expanded. A prospective multihospital study is under way.

Our findings are in accord with those of Persoglia and Maiolo (*4*), who found noncancerous multinucleated transitional epithelial cells in the urine of 21 cases of carcinoma of the cervix.

We cannot at present explain our results but can analyze them in relation to modern theories on the etiology of malignant disease. If human tumors are caused by viruses (*5*) it is possible that such viruses could cause multinucleation in distant epithelial tissues. Nowakovsky *et al.* (*6*) have already shown that the herpes simplex virus produces epithelial multinucleation not only in the original lesion but also in apparently unaffected tissues such as the transitional epithelium of the urinary collecting system.

According to Green, Bennett, Davidsohn, and Eilber and their co-workers (*7*), malignancy is associated with immunologic changes. It may be, therefore, that the phenomena we have described are related to disturbances in humoral activity in patients with cancer. Berkheiser (*8*) has shown that corticosteroids, which have a strong immunosuppressive action, produce hyperplasia of the tracheobronchial epithelium.

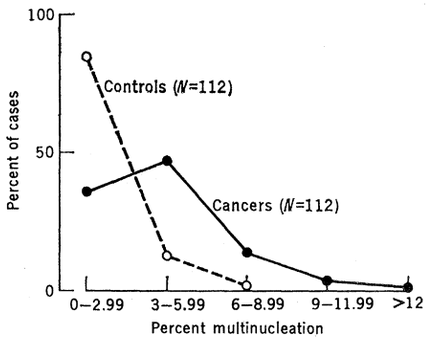

Fig. 1. Frequency distribution of multinucleated ciliated tracheobronchial epithelial cells in patients suffering from known malignancies, compared with a matched control group.

It may be that patients with carcinoma have an inherent tendency to multinucleated cells or their production by some invasive or inherent mechanism.

An important aspect of our study is the search for occult cancer in patients with high percentages of tracheobronchial epithelial multinucleation in the control group.

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Paradoxical Increase in Rate of Catabolism of Low-Density Lipoproteins after Hepatectomy

Abstract. *It has been suggested that the liver may be a major site for irreversible degradation of low-density lipoprotein (LDL). The disappearance of autologous ^{125}I-labeled LDL from plasma was compared in intact and in hepatectomized swine. Contrary to expectations, the rate of irreversible removal of LDL from plasma was increased rather then decreased by hepatectomy. These studies suggest that the liver is not a major site for LDL removal. We propose further that the liver (or some function requiring an intact liver) may affect the metabolism of LDL in a manner that prolongs its lifetime in the plasma compartment.*

Because the levels of low-density lipoprotein (LDL) in plasma are implicated in the pathogenesis of atherosclerosis, it is important to understand the mechanisms regulating LDL levels. It is generally accepted that the liver is the primary site of LDL biosynthesis although evidence for production of LDL apoprotein by the intestine has been reported (*1*). Very little is known about the sites or the mechanisms for LDL removal from plasma. Hotta and Chaikoff (*2*) reported that the rate of disappearance of labeled cholesterol from rat plasma was sharply reduced or arrested by hepatectomy. From this they concluded that the liver was the site of both the origin and the degradation of plasma lipoprotein cholesterol. Lewis *et al.* (*3*) reported a decrease in lipoprotein levels in hepatectomized dogs and suggested that there was some metabolism of lipoproteins in the periphery. Hay *et al.* (*4*), following the fate of [^{125}I]LDL, have suggested that in the rat the liver is a major site of LDL catabolism. We have previously shown that the major extravascular pool of LDL in swine lies in the liver (*5*). After injection of [^{125}I]LDL the liver

contained an amount of trichloroacetic acid–precipitable radioactivity corresponding to 10 to 15 percent of that in the plasma when the animals were killed, and this was relatively independent of the duration of the study (8 to 122 hours). This finding is also compatible with the suggestion that the liver plays a major role in irreversible catabolism of LDL. Were this so, hepatectomy in swine should arrest or markedly reduce the rate of removal of LDL. As shown below, this is not the case; the rates of removal of LDL actually increase after hepatectomy.

Blood was drawn from donor swine (Hormel white or miniature) weighing 30 to 50 kg. Low-density lipoprotein (density, 1.019 to 1.063 g/ml), isolated by preparative ultracentrifugation (*6*) and recentrifuged at a density of 1.063, was reacted with ^{125}ICl by a modification of the method of McFarlane (*7*). After dialysis against a mixture of 0.01 percent ethylenediaminetetraacetate and 0.9 percent NaCl, the [^{125}I]LDL was reisolated by ultracentrifugation at a density of 1.063 and was sterilized by passage through a Millipore filter (pore size, 0.45 μm).

Experimental animals were fasted overnight and catheters were inserted in both external jugular veins. A tracer dose of [^{125}I]LDL (less than 10 mg of LDL protein) was injected; plasma samples were obtained at frequent intervals and radioactivity was measured in a deep-well scintillation counter to an accuracy of ± 2 percent. The validity of the use of [^{125}I]LDL prepared in this way has been documented in several ways. (i) Less than 2 percent of the radioactivity was in the lipid moiety [extractable with a mixture of chloroform and methanol (2 : 1)] and more than 98 percent was precipitable with trichloroacetic acid. (ii) All of the ^{125}I in labeled preparations migrated on agarose gel electrophoresis together with unlabeled swine LDL. (iii) Reisolation of LDL from plasma at various intervals after injection showed that 95 percent or more of the plasma ^{125}I remained in the LDL fraction. (iv) The labeled preparations did not contain significant amounts of denatured lipoproteins that might be rapidly removed from the plasma. This was shown in several experiments by agreement between the total dose of ^{125}I injected and the calculated total ^{125}I distributed at time zero in the plasma volume (measured by [^{14}C]dextran injection just before the animals were killed). (v) The characteristic biphasic curve of disappearance seen in all the primary recipients (Fig. 1) was reproduced when plasma drawn at 4 hours from the primary recipient was injected into a second recipient animal ("biological screening"). (vi) As shown below, after hepatectomy the rate of disappearance of total LDL protein paralleled that of [^{125}I]LDL.

Six days after the control study, the animal was anesthetized with sodium thiopental and the liver was removed en bloc. Because the vena cava in swine passes through the body of the liver and had to be interrupted, venous return from the viscera was maintained by introducing a three-way Dacron graft connecting the portal vein and the cut ends of the vena cava. Blood glucose was maintained with a continuous infusion of glucose (approximately 15 g/hour) in 0.45 percent NaCl. Physiologic status was monitored and remained stable over the study period of 16 to 20 hours (blood pressure, blood glucose, hematocrit, pH, pO_2, pCO_2). Postmortem examination showed no significant blood in the thorax, abdomen, or gastrointestinal tract. The blood drawn for analyses never exceeded 7 percent of the total blood volume. Blood volume determined in two hepatectomized swine ([^{14}C]dextran space) at the end of the study was within normal limits. During the studies made after hepatectomy, the total protein content of LDL reisolated at a density of 1.006 to 1.063 was determined (*8*). Plasma albumin levels determined in two animals did not change over the period of the study.

The disappearance curves from the control studies were resolved into two exponential components using least-squares fitting procedures. The average correlation coefficients for the least-squares regression lines were $r = .974 \pm .008$ standard error of the mean (S.E.M.) for the first phase and $r = .984 \pm .008$ for the second phase. The half-lives of the first (faster) and second (slower) exponentials were determined from their slopes (b_2 and b_1). The fractional catabolic rate (*FCR*) was calculated from the slopes and the zero time intercepts (C_2 and C_1) by using the following equation (*9*):

$$FCR = \frac{1}{C_1/b_1 + C_2/b_2}$$

After hepatectomy, strictly monoexponential disappearance curves were observed and fitted by single least-squares regression lines. The average correlation coefficient for these was $r = .995 \pm .001$. Since the monoexponential disappearance curve after hepatectomy is consistent with a single compartment model, the fractional catabolic rate of plasma LDL is given directly by the observed slope of the plasma ^{125}I disappearance curve.

Representative [^{125}I]LDL disappearance curves from one animal before (A) and after (B) hepatectomy are shown in Fig. 1. The decay curves in the intact animals could all be resolved into two exponential components as described above. The observed half-lives for the rapid component (phase 1) and the slow component (phase 2) in five experiments are shown in Table 1 together with the calculated fractional catabolic rates. The mean half-life for phase 1 was 54.6 ± 3.6 minutes and for phase 2 it was 19.3 ± 0.7 hours; mean fractional catabolic rate was 4.57 percent of the total plasma LDL per hour.

After hepatectomy, the plasma decay curve was in every case monoexponential as in Fig. 1. The observed half-lives for plasma radioactivity after hepatectomy are considerably shorter than the phase 2 half-lives before hepatectomy, as shown in Table 1. Some decrease in the observed half-life of isotope disappearance would be expected simply as a result of removing a large fraction of the extravascular exchanging pool. The observed slope of the second exponential in the intact animal is in fact less than the true fractional rate of irreversible removal from the plasma compartment. The critical datum is the fractional catabolic rate defined above, which reflects irreversible catabolism. As shown in Table 1, after hepatectomy

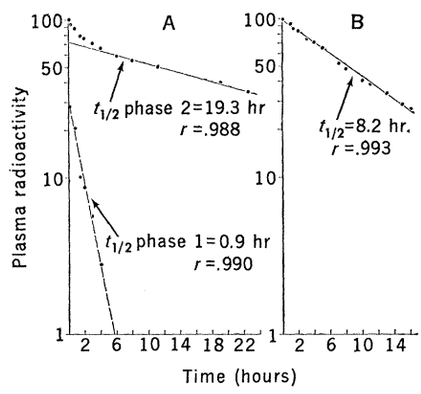

Fig. 1. Representative curves for disappearance of plasma radioactivity after injection of [^{125}I]LDL. (A) Before hepatectomy (pig 1). The lower curve was obtained by subtracting the extrapolated second exponential phase values from the experimental values shown above. (B) After hepatectomy (pig 1).

Table 1. Comparison of LDL turnover in swine before and after hepatectomy.

Before hepatectomy			After hepatectomy		
Observed half-life of plasma radioactivity		Calculated fractional catabolic rate of plasma LDL* (per hour)	Observed half-life of plasma radioactivity (hours)	Observed half-life of total LDL protein† (hours)	Calculated fractional catabolic rate of plasma LDL‡ (per hour)
Phase 1 (minutes)	Phase 2 (hours)				
64	17.1	0.0466	9.9	9.6	0.0700
52	19.6	.0439	11.0	11.4	.0632
52	19.3	.0471	9.0	9.3	.0773
44	19.3	.0474	9.8	10.2	.0705
61	21.3	.0436	6.9	7.2	.1010
Mean 54.6	19.3	.0457	9.3	9.5	.0764
S.E.M. 3.6	0.7	.0008	0.7	0.7	.0065

* Calculated from $FCR = 1/(C_1/b_1 + C_2/b_2)$, where C_2 and C_1 are the intercepts of the first (faster) and second (slower) exponential components, and b_2 and b_1 are the slopes (*8*). † Half-life derived from direct measurement of *net* LDL protein concentration as a function of time. ‡ Calculated from the slope (on semilogarithmic plot) of disappearance curve of plasma radioactivity, which was monoexponential.

this increased from 4.6 to 7.6 percent per hour ($P < .02$), an increase of 67 percent. During the studies after hepatectomy, total LDL protein levels fell progressively and, as shown in Table 1, the rate of disappearance of LDL protein was, within experimental error, the same as that for disappearance of ^{125}I. Preliminary studies in three dogs have yielded similar results, fractional catabolic rate increasing from 0.030 ± 0.001 to 0.062 ± 0.001 per hour (*10*).

These results in hepatectomized animals strongly support the conclusions drawn from tissue distribution studies that the liver represents the major extravascular pool of LDL. The monoexponential disappearance after hepatectomy suggests that other extravascular pools exchanging with the plasma LDL pool must be either very small or very slow to equilibrate with plasma LDL.

The failure of hepatectomy to arrest or even to slow the disappearance of LDL from the plasma compartment was quite unexpected. This finding does not necessarily rule out some degree of LDL catabolism by the normal liver. However, if this represented an important fraction of the total LDL catabolism it would be anticipated that some decrease in fractional catabolic rate would be observed on removal of the liver. Because the liver is the only major site for cholesterol breakdown and because LDL is the major carrier of plasma cholesterol in swine, man, and certain other species, there has been a tendency to assume that the liver would be the major site of LDL removal from the plasma. Hotta and Chaikoff (*2*) drew such a conclusion from their studies in rats. What they demonstrated was that the specific radioactivity of plasma cholesterol did not decline further when the liver was excluded from the circulation. Changes in the concentration of total plasma cholesterol were not reported and there may have been a decline in cholesterol concentration without change in specific radioactivity, that is, influx of unlabeled cholesterol may have been arrested without arresting efflux of cholesterol from the plasma. This is in fact what was observed in the present studies with regard to LDL apoprotein—there was a progressive fall in the concentration of LDL protein with no change in its specific radioactivity. Thus, we can also conclude from the present studies that, under the conditions used, tissues other than the liver do not contribute importantly to steady state concentrations of LDL in the plasma.

Finally, it is necessary to explain the apparent increase in fractional rate of LDL removal after hepatectomy. Anesthesia itself does not account for it; several of the intact animals were studied under similar anesthesia and one pig was subjected to removal of the entire small intestine without effect on the LDL disappearance curve. Even though the major physiologic variables monitored during the study remained stable, there may be progressive biochemical perturbations that could directly or indirectly influence LDL catabolism not necessarily relevant to regulation under physiological conditions. For example, there may be accumulation in the plasma of metabolites that destabilize LDL. Arguing against this explanation is the fact that the rate of disappearance was first-order and constant from the time of injection, which was done immediately after hepatectomy. If accumulation of a metabolite were responsible, one might have anticipated a progressively faster rate of removal as the study progressed, but this was not observed. An alternative hypothesis is that the liver itself, or some function dependent upon the presence of an intact liver, plays a role in the metabolism of LDL so as to lengthen its lifetime in the plasma-liver compartment. For example, LDL may acquire increments of lipid in the periphery that modify it and shorten its lifetime unless that lipid is removed by the liver (or through the action of an enzyme produced by the liver). Whether such a postulated "repair" process is peculiar to LDL or whether it applies in the case of other transport proteins remains to be determined.

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