Table 1. Amount of incorporation (disintegrations per minute) of [^{3}H]ATP into RNA and DNA.

Substance	Incorporation	Percent of total
Total (DNA + RNA)	319,000	
RNA	237,264	95.0
DNA	12,560	5.0
% Recovery		77.5

at 90°C for 30 minutes. The radioactivity of DNA was assessed as described for RNA.

Quinacrine has a marked effect on the uptake of [8-^{3}H]adenosine into cells parasitized by *P. berghei* at 10^{-4} and $10^{-5}M$. Results of others indicate that rat red cells contain the enzyme adenosine kinase (*5*). Our data, showing inhibition of adenosine transport by various drugs, suggest that this enzyme is membrane bound (*2*). Other observations indicate that this adenosine transport is an active, concentrating mechanism probably concerned with phosphorylation of adenosine to adenosine monophosphate (*6*). That this is reasonable is shown by the fact that once adenosine penetrates the red cell it does not wash out of these cells; and chromatography indicates that the radioactivity is associated with adenosine monophosphate (AMP). Similar observations are reported in other cell types (*5*); and acridine derivatives, such as quinacrine, inhibit adenosine transport in these cells (*7*). Owing to deficient de novo synthesis of purines, the malarial parasite is dependent on exogenously supplied purines (*4, 8*), and any inhibition of the transport system might constitute an indirect "starvation" mechanism at the membrane of the host cell. Also if transport into the parasite of phosphorylated or nonphosphorylated purine intermediates could be inhibited, this would represent an important new antimalarial mechanism. There does appear to be a relation between host red cell ATP and degree of parasitemia (*9*).

The really surprising finding, however, was the selective effect of various doses of quinacrine on the incorporation of [^{3}H]adenosine triphosphate into RNA of erythrocyte-free malarial parasites (Fig. 2) compared to the control (Table 1). The same results occurred if deoxyadenosine was used (*10*). On the basis of quinacrine's interference with the incorporation of adenosine triphosphate into DNA, several workers using polymerases from *Escherichia coli,* (*11*) have predicted the same inhibition by quinacrine in malarial parasites. However, the fact that much more incorporation occurred into RNA (10 or 20 times as much) than into DNA and that this incorporation is drug-inhibited to a greater extent shows that there is a surprising selectivity of drug action. This would possibly indicate that, in parasites, there is some basic difference in the nucleic-acid synthesizing system of parasites. There is some factual basis for this statement because ion requirement of the parasite enzyme is different—Co++ and Mn++ stimulate more than Mg++—and maximum activity occurs at *p*H 7.4, compared to *p*H 8.0 for the corresponding mammalian enzyme (*10*). Also Rifampicin (a direct inhibitor of bacterial RNA polymerase) is effective against malarial parasites in vivo (*12*).

Many of the studies with antimalarial drugs have been made with organisms other than malarial parasites (*13, 14*) and in subcellular systems rather than in more complex systems (*15*). Our data with various concentrations of quinacrine mirror the effects of the in vitro polymerase system quite well (*13*), but we think that the selective toxicity of quinacrine's action may well lie in the nucleic acid synthesizing system. Probably the interference of quinacrine's action with the RNA polymerase is indirect by way of "intercalation" in the DNA template (*16*). We have actually observed, using fluorescence microscopy, the direct result of quinacrine's intercalation. Parasitized cells were placed in the presence of quinacrine, under ultraviolet light excitation, and the parasite nuclei showed yellow-green fluorescence. This well-characterized effect has been developed both in parasitized cells with acridine orange (*17*) and in vitro with ethidium bromide (*18*). Both of these drugs are well-known intercalating compounds (*19*).

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27 March 1970

Immunoassay of Plasma Low-Density Lipoproteins

Abstract. *An immunoassay was developed for determining the concentration of the protein moiety of the low-density lipoproteins of human plasma. The concentration of this protein in the plasma was variable; it was higher than normal on the average in patients with familial hyperbetalipoproteinemia (type II) and endogenous hyperlipemia (type IV) and lower than normal in patients with fat-induced (type I) and mixed (type V) hyperlipemia. Patients with endogenous hyperlipemia were separable by the immunoassay into those with normal and those with supernormal low-density lipoprotein protein concentration.*

Low-density lipoproteins (LDL) have been statistically associated with coronary heart disease (*1*). They are usually quantitated by their cholesterol content or total mass (*1–4*), although variability in the relative proportions of their several components has been recognized for some years (*5*). In light of the evidence (*5*) that the cholesterol and protein content of LDL might vary independently of each other, the concentration of LDL protein was determined in plasma of normal and hyperlipidemic subjects and compared with LDL cholesterol concentration.

Blood was drawn from 165 subjects

Table 1. Comparison of LDL protein with LDL cholesterol concentration in normal subjects and in hyperlipoproteinemia.

Type	Subjects (No.)	Sex (M/F)	Mean age (years)	LDL protein ± S.D. (mg/100 ml)	LDL cholesterol ± S.D. (mg/100 ml)	C/P ± S.D.
Normal	64	32/32	34	83 ± 25	140 ± 32	1.7 ± 0.4
I	3	2/1	25	*40 ± 8	†28 ± 10	†0.7 ± 0.2
II	44	19/25	46	*129 ± 43	†236 ± 67	1.8 ± 0.5
III	15	13/2	51	71 ± 48	154 ± 120	†2.2 ± 0.4
IV	17	11/6	49	*103 ± 38	135 ± 41	†1.3 ± 0.3
V	22	17/5	42	75 ± 47	†78 ± 48	†1.0 ± 0.5

* Significantly different from age-matched normal subjects ($P < .05$). †Significantly different from age-matched normal subjects ($P < .001$).

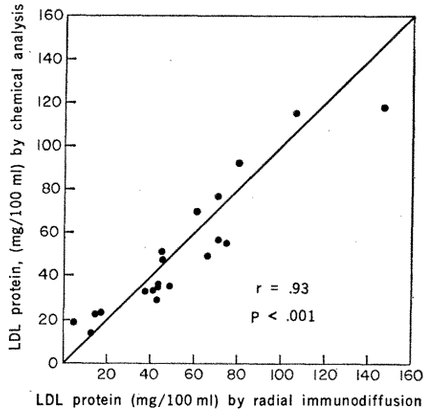

Fig. 1. Correlation of radial immunodiffusion assay with chemical analysis of LDL protein. Twenty LDL samples from normal and hyperlipidemic subjects were analyzed by both methods simultaneously. Similar results were obtained by the two techniques.

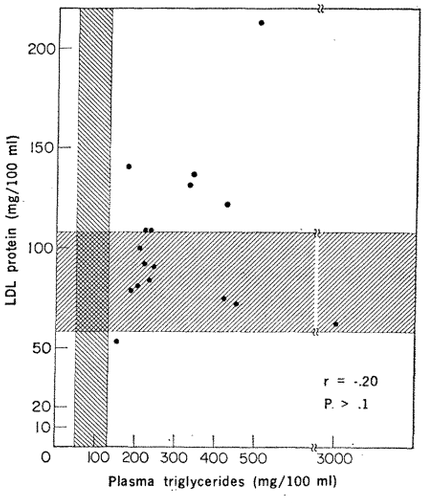

Fig. 2. Lack of correlation of LDL protein concentration with plasma glycerides in type IV hyperlipoproteinemia. In this syndrome, the major plasma lipid abnormality is hyperglyceridemia. Measurement of LDL protein in plasma permits subdivision of this heterogeneous group of patients into two subgroups, with LDL protein concentrations higher or lower than the normal mean. The normal mean and standard deviation for plasma glycerides and for LDL protein are indicated on the graph by cross-hatching.

(94 males and 71 females) who had fasted for 12 to 16 hours. Most of the subjects were on a free diet. Measurement of plasma cholesterol and glycerides, lipoprotein electrophoreses, and phenotyping of hyperlipoproteinemia were performed as described (*3*, *4*). The concentration of LDL cholesterol was determined chemically (*4*), and LDL protein was measured by radial immunodiffusion (*6*); chylomicrons and very-low-density lipoproteins were removed before analysis by ultracentrifugation at 140,000*g* for 16 hours without density adjustment (*4*, *7*).

To ascertain that the immunoassay measured LDL protein rather than total lipoprotein or any other component of LDL, LDL was isolated from samples of plasma (*8*) from 20 subjects by sequential ultracentrifugation at a density of 1.006 and at 1.063. Chemical measurements of cholesterol (*4*) and protein (*9*) were performed on the isolated LDL. Immunoassay results correlated with both cholesterol ($r = .85$) and protein ($r = .93$) determinations. Since cholesterol and protein content of the lipoprotein were related to each other ($r = .89$), it was necessary to perform partial correlation analysis to determine which component correlated best with the immunoassay. The multiple correlation coefficient (*10*) of the immunoassay with cholesterol and protein was .99. Other parameters of LDL composition, such as glyceride, phospholipid, or water content, did not, therefore, affect the immunoassay. The partial correlation coefficient between LDL cholesterol concentration and the immunoassay, with protein fixed, was small and apparently insignificant ($r = .14$), while LDL protein and the immunoassay, with cholesterol fixed, were highly correlated [$r = .72$ ($P < .05$)]. Thus, the radial immunodiffusion technique appears to be a valid method for measurement of LDL protein (Fig. 1).

The concentration of LDL protein in normal subjects was 83 ± 25 mg/100 ml (Table 1). In patients with hyperlipoproteinemia, LDL protein concentration varied much more widely.

To relate these values to the more familiar LDL cholesterol concentration, the ratio of cholestrol to protein (C/P) in LDL was calculated. This ratio in normal and in type II subjects (Table 1) is in agreement with that obtained by other methods (*5*, *11*). In the lipemic disorders, types I, III, IV, and V, all of which are characterized by abnormalities in the very-low-density lipoprotein spectrum (*3*), LDL appears to be abnormal in composition, as evidenced by abnormal C/P ratios. These data are consistent with the differences in LDL flotation rate in the ultracentrifuge which have been shown to occur between normal subjects and patients with hyperlipoproteinemia (*12*).

Abnormalities in LDL protein concentration are usually accompanied by abnormalities in LDL cholesterol concentration (Table 1). In type IV, however, LDL cholesterol concentration is normal, but LDL protein is high. Furthermore, LDL protein concentration appears to vary independently of plasma glyceride concentration in type IV (Fig. 2). It is possible that type IV patients with elevated LDL protein are clinically distinct from those with low or normal LDL protein. This distinction may have prognostic value for estimating risk of coronary heart disease and response to therapy with hypolipidemic drugs.

Since LDL protein is in part a product of VLDL catabolism (*13*), and its removal from plasma appears to follow first-order kinetics (*6*), its concentration is presumably an indirect measure of VLDL transport through plasma. Its elevated concentration in many type IV hyperlipoproteinemic patients, who commonly have coronary heart disease but whose plasma and LDL cholesterol concentrations are often normal (*14*), suggests that it could be a better predictor of coronary disease risk than either of the other measurements.

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7. Since chylomicrons and VLDL share common antigenic determinants with LDL (*3*, *4*), it is essential to remove them before LDL immunoassay. This is conveniently done without altering LDL concentration as outlined in (*4*, p. 31).
8. This group included 9 samples from men and 11 from women. Four of the plasma samples were from normal subjects, 1 each from subjects with types I, II, and III, 5 from subjects with type IV and 7 from those with type V lipoprotein patterns. The group was so selected in order to have representation of all levels of plasma glycerides from $<$ 50 to $>$ 5000 mg/100 ml.
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Chromosomal Abnormalities in the Human Population: Estimation of Rates Based on New Haven Newborn Study

Abstract. *The incidence of gross chromosomal abnormality was measured in a large (4500), relatively unbiased sample of New Haven infants born during 1 year. The frequency of infants with abnormal chromosomal constitutions was 0.5 percent. For mothers over age 34, 1.5 percent of newborns were chromosomally abnormal. Only one in four of these infants could have been detected by phenotypic criteria alone. Methods are discussed whereby this fraction of the newborn population might be detected and possibly reduced.*

The genetic, clinical, and social significance of chromosomal variation in the human population can only be determined by studying a large, unbiased sample. Since it is now possible to determine the karyotypes of fetuses by amniocentesis during the 12th to 16th week of pregnancy (*1*), it is extremely important to know the significance of chromosomal variation. Most cytogenetic studies have been carried out on institutionalized patients or other small, biased populations. The present study is the largest, relatively unbiased survey of the chromosome complements of newborns yet performed. Forty-five hundred infants, born consecutively over 1 year at Yale–New Haven Hospital, were studied. The New Haven area population is approximately 400,000, and the total number of births each year is about 6900. The remaining 2400 babies in the area are born at a second hospital. The major difference between the two neonatal populations is that a larger percentage of Negro infants were born at Yale–New Haven Hospital (18 percent) than were born at the Hospital of St. Raphael (13 percent).

Leukocytes from the cord blood of each infant were grown in vitro and then prepared for cytological examination by methods described elsewhere (*2*). Two cells from each infant were photographed and then idiogrammed according to standard conventions (*3*). In instances where abnormalities were detected, 30 cells were photographed and studied. Complete clinical and sociological data were recorded for each infant and its family. Chromosomal, clinical, and sociological data were coded for computer storage in order to facilitate information retrieval and analysis (*2*).

Table 1. Number of chromosome abnormalities.

Type of abnormality	Chromosomal abnormalities	
	No.	No./1000 births
Translocations	6	1.37
Trisomy D	1	0.23
Trisomy E	1	0.23
Trisomy G	3	0.69
XYY	3	0.69
XXY	4	0.92
XXX	3	0.69
XO	1	0.23

Successful chromosome preparations were obtained from 4366 of 4482 infants included in the study (97.4 percent). Twenty-two infants had a chromosome abnormality (Table 1). Half involved the sex chromosomes and half the autosomes. The overall frequency (1/200) is comparable to other surveys of consecutive newborns (*4*, *5*). Similarly, in these other studies the abnormalities were divided equally between the autosomes and the sex chromosomes. The frequency of 1/200 is a minimum estimate, since our study is based upon a sample of only two cells per newborn and thus does not include mosaics.

Six infants had a translocation, and five had an autosomal trisomy (Table 2). The frequency of XYY karyotypes in male infants was 3 in 2184 in our study, 5 in 3496 in the Edinburgh study (*5*), and 4 in 1066 in the Ontario study (*4*). If these studies of consecutive newborns are pooled, an XYY karyotype occurred once in 570 male births. In a survey of more than 2000 male newborns in Boston (*6*), most of whom were selected because of a normal phenotype, no XYY males were found. Although the frequency of XYY males would be lower if these newborns were included in the calculation, the latter series is not comparable and therefore cannot be considered together with the surveys of consecutive newborns. Whether the variation in frequency of an XYY karyotype in surveys of newborns is due to the relatively small size of each of the surveys or represents a real epidemiological difference is currently unclear. In the present study, an XXY karyotype was present in 1 of 545 male babies. An XXX karyotype occurred once in 727 female newborns, and a 45, X karyotype only once in 2181 female newborns. Trisomy G in our study was less common than reported previously (*7*, p. 150). The maternal age of 25.7 years in the New Haven population was about 2 years lower than that for the total United States, England, and Australia in recent years (*7*, p. 157), and there were only half as many mothers over 34 years in the present study (7.7 percent). The lower incidence of Trisomy G (1/1455) in our study may represent a real difference from the reported frequency of 1 in 600 to 700 in the literature because of the lower maternal age. A similar downward trend in the incidence of Trisomy G in Victoria and England has been reported recently by Collmann and Stoller (*8*).

Six infants were found to have trans-