thranilic oxidase in these livers, it may be concluded that the failure of diabetic animals to synthesize nicotinic acid is not caused by a decrease in any essential enzyme, but must be attributed to some other type of control mechanism. The striking increase in picolinic carboxylase, from immeasurably low concentration to appreciable concentration, has previously been correlated with the decreased production of nicotinic acid. The decrease in quinolinic acid formation caused by an increase in picolinic carboxylase activity provides an adequate explanation for the control of nicotinic acid biosynthesis.

The physiological significance of this phenomenon remains obscure, since it is not clear what advantage is gained by the diabetic organism through diverting an intermediate from nicotinic acid synthesis. The mechanism by which hormonal regulation of picolinic carboxylase operates is also not known. It does seem clear, however, that the more general phenomenon of competition for available substrate by different enzymes is exhibited in an extreme form in this system, in which the substrate for a spontaneous reaction is utilized by an enzyme whose activity changes from very low to higher values.

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3 June 1964

Nucleic Acid Metabolism in L Cells Infected with a Member of the Psittacosis Group

Abstract. *Multiplication of the meningopneumonitis agent in the cytoplasm of L cells in suspension culture is accompanied by parallel increases in cytoplasmic DNA, RNA, and phospholipid. Synthesis of RNA and phospholipid in infected cells is inhibited at the height of meningopneumonitis multiplication, but nuclear DNA synthesis is inhibited earlier and to a greater degree.*

Members of the psittacosis group are obligate intracellular parasites that grow in the cytoplasm of their host cells. Purified suspensions of these infectious agents contain approximately equal amounts of DNA and RNA (*1*), but information about the kinetics of DNA and RNA synthesis in the agent, and the effects of multiplication of the parasite on the nucleic acid metabolism of the host cell, is meager and contradictory. The total DNA and RNA content of infected cell cultures increases as the infectious titer rises (*2, 3*). It was concluded from studies with the agent of psittacosis that DNA synthesis begins early (*4*) but that there is a 10-hour lag between synthesis of DNA and its incorporation into particles (*5*), and another investigation with the trachoma agent indicated that DNA synthesis is most active late in the infection while RNA synthesis occurs at an earlier stage (*3*).

To gain a clearer picture of the nucleic acid metabolism of cells infected with the agent of meningopneumonitis (Cal 10 strain), a well-characterized member of the psittacosis group (*1, 2*), we determined the rate of DNA and RNA synthesis by measuring the uptake of orthophosphate labeled with P^{32}. The cells were exposed to the isotope for brief periods at various times after infection. Labeled cells were separated into nuclear and cytoplasmic fractions, and the cytoplasm was further separated into supernatant and sediment. In this way, synthesis of agent nucleic acid in the cytoplasm could be differentiated from synthesis of host nucleic acid in the nucleus. Synthesis of agent DNA and RNA was clearly evident after 10 to 15 hours of infection and was at a maximum after 20 to 25 hours, the period of highest multiplication rate. The rate of host RNA synthesis was significantly reduced only at the time of maximum agent multiplication, but the rate of host DNA synthesis was profoundly inhibited as early as 10 to 15 hours, when agent multiplication had only just begun.

Mouse fibroblasts (strain L cells) exponentially multiplying in suspension cultures were infected with a strain of meningopneumonitis agent well adapted to L cells. As measured by microscopic count of inclusions, 40 to 70 percent of the cells were infected. The growth medium was M-199 containing 16 percent fetal calf serum, and streptomycin. Uninfected suspensions of L cells were included in each experiment. At 5-hour intervals after infection, 0.67 mc P^{32} as carrier-free orthophosphate was added for each 3 to 5 $\times$ 10^7 cells. Exactly 5 hours after the addition of P^{32}, the cells were collected by centrifugation, washed three times in Hanks balanced salt solution (0.01*M* in phosphate) containing 0.15 percent Methocel to preserve cell integrity, and separated into nuclear and cytoplasmic fractions by shaking at 4°C for 1 hour with 20 percent citric acid and then for one-half hour with 30 percent citric acid. Less than 2 percent of the P^{32}-labeled DNA in uninfected cells appeared in the cytoplasmic fraction. The cytoplasmic fraction was further separated into supernatant and sediment by centrifuging at 5000*g* for 30 minutes, just sufficient to sediment the meningopneumonitis agent. The cytoplasmic sediment was washed twice with balanced salt solution containing Methocel. The phosphorus of each fraction was partitioned by the Schneider-Schmidt-Thannhauser technique (*6*) into acid-soluble P, lipid P, RNA P, DNA P, and residue P, and the P^{32} content of each part was determined in a thin-window counter. On the assumption that the uninfected cells in an infected culture were metabolizing at a normal rate, the data were corrected for the contribution of uninfected cells

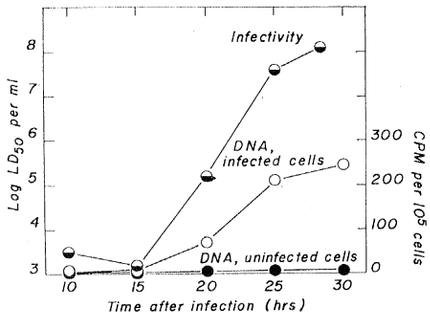


Fig. 1. Cumulative uptake of P^{32} by the DNA of the cytoplasmic sediment of infected and uninfected L cells compared with multiplication of the meningopneumonitis agent. Cell-associated infectivity was determined as the LD_{50} (50 percent lethal dose) for the chick embryo yolk sac.

and finally expressed as counts per minute per 10^5 infected cells.

The DNA and RNA content of uninfected L-cell suspensions agreed with previously published values (*7*), and the incorporation of P^{32} into DNA varied only slightly during different periods of labeling, indicating that a constant fraction of the L-cell population was entering into DNA synthesis at any given time. The pattern of multiplication of the meningopneumonitis agent in suspensions of L cells closely resembled that described for the same host–agent system by Higashi *et al.* (*3*).

Figure 1 shows that significant incorporation of P^{32} into the DNA of the cytoplasmic sediment occurred only in infected cells. Therefore, this uptake probably represents synthesis of agent DNA. Synthesis of cytoplasmic DNA with a sedimentation behavior characteristic of intact particles of meningopneumonitis was first detectable after 10 to 15 hours of infection, about the time rapid multiplication began. It was at a maximum after 20 to 25 hours, the period of greatest increase in meningopneumonitis infectivity.

In contrast to DNA, newly synthesized RNA and phospholipid [also a major phosphorus fraction in tthe meningopneumonitis agent (*1*)] appeared in appreciable quantities in the cytoplasmic sediment of both uninfected and infected cells. Therefore, the effect of infection on incorporation of P^{32} into the RNA and phospholipid was not as obvious as the effect on DNA, but there was still a considerable rise in P^{32} uptake by both these fractions (Fig. 2). As with DNA, the increased rate of incorporation due to agent growth was first apparent during the 10- to 15-hour labeling period and was at a maximum during the 20- to 25-hour labeling period.

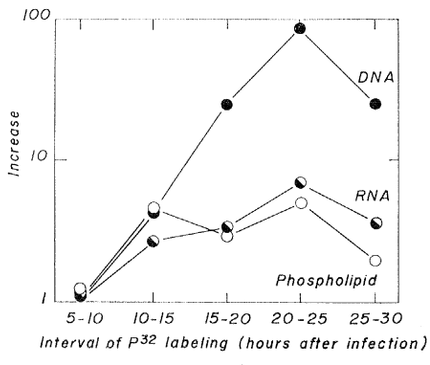
Figure 3 shows that infection with the meningopneumonitis agent had no effect on the incorporation of P^{32} into the DNA of host nuclei for the first 10 hours after infection. Then, beginning with the 10- to 15-hour labeling period, there was almost complete inhibition of uptake of label into the nuclear DNA fraction. The amount of P^{32}-labeled DNA in the cytoplasmic supernatant rose slightly at 10 to 15 hours and remained relatively constant thereafter, suggesting that some DNA had leaked out of the nuclei of infected cells. However, this apparent leakage was not nearly sufficient to account for the decreased amount of labeled DNA in these nuclear fractions.

The value for P^{32} uptake by the nuclear DNA of each of ten suspensions of infected cells was almost zero, when corrected for the uptake expected for the varying numbers of uninfected cells present (40 to 70 percent). This indicates that correction for the contribution of uninfected cells is valid; that is, they are not affected by the presence of infected cells. It thus seems probable that the uninfected cells in infected suspensions were synthesizing DNA at normal rates while the infected cells were making practically none at all.

In contrast to the uptake of P^{32} by the DNA of the nucleus of the infected cell, incorporation into the RNA and phospholipid fractions was not significantly inhibited until 20 to 25 hours after infection (Fig. 3).

In agreement with previous reports (*4*, *5*), we observed that the synthesis of DNA by the meningopneumonitis agent began 10 to 15 hours after infection and continued throughout the period of observation. However, we obtained no evidence for a lag between DNA synthesis and appearance of infectious particles. We agree with the conclusion (*3*) that most of the DNA is made late in the infection (in our system, at 20 to 30 hours) but we found no temporal dissociation of RNA and DNA production. Our results suggest that synthesis of meningopneumonitis DNA, RNA, and phospholipid occurs simultaneously and that synthesis of these agent components is closely correlated in time with the formation of new agent particles.

The striking inhibition of DNA synthesis in the nuclei of infected cells in suspension culture is not in accord with radioautographic studies with infected cell monolayers in which no (*8*) or only moderate (*9*) inhibition of nuclear uptake of tritiated deoxynucleosides was observed. Perhaps these divergent results may be partially explained by the greater lethality of psittacosis agents for L cells in suspension cultures than in monolayers growing on glass. As first observed by Higashi *et al.* (*2*) and confirmed in these studies, L cells in suspension culture began to die about 20 hours after infection and were nearly all dead by 30 hours, a much more rapid cytopathic effect than was observed in monolayers. It is of interest that vaccinia virus more strongly inhibited host DNA synthesis in cell suspensions than in monolayers (*10*). The inhibition of nuclear RNA and phospholipid turnover at the height of agent multiplication may logically be explained in terms of competition between host and parasite for synthetic inter-



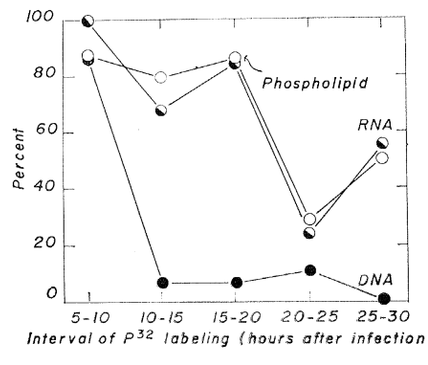


Fig. 2 (left). Uptake of P^{32} by phosphorus-containing fractions of the cytoplasmic sediment of infected cells, expressed as multiples of the uptake by uninfected cells. Fig. 3 (right). Uptake of P^{32} by phosphorus-containing fractions of nuclei of infected cells, expressed as the percentage of uptake by the nuclei of uninfected cells.

mediates. However, inhibition of DNA synthesis begins at a time when the mass of the parasite is so small as compared to the host that effective competition seems unlikely, and another mechanism must be sought.

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Macroglobulin from Human Plasma Which Forms an Enzymatically Active Compound with Trypsin

Abstract. *The protein from human plasma which forms an enzymatically active complex with trypsin and is able to protect trypsin from inhibition by soybean trypsin inhibitor has been shown to be an α_2-macroglobulin. It was obtained from the lipid-poor euglobulin of Cohn Fraction III-0, and appears to be closely related to the 19S-glycoprotein or α_2-macroglobulin.*

Haverback *et al.* (*1*) have reported a protein in human plasma which combines with trypsin or chymotrypsin in such a way that the enzyme is active but can no longer be inhibited by certain inhibiting proteins, such as that from soybean. The trypsin-binding activity and a major component in preparations obtained by chromatography of plasma on diethylaminoethylcellulose (*2*) had a sedimentation coefficient between 15*S* and 20*S*. Since Haverback and associates had already shown that this component is an α_2-globulin, it appeared that the activity might reside in the α_2-macroglobulin, also known as 19*S*-α_2-glycoprotein (*3–6*). Consequently, we have prepared α_2-macroglobulin from Cohn Fraction III-0 by a modification of the procedure of Brown *et al.* (*4*) and tested this for trypsin-binding activity.

Fraction III-0 was prepared in the Hyland Laboratories (*7*) from a pool of fresh human plasma by Cohn methods 6 and 9 (*8*). The III-0 paste was kept in dry ice until dialyzed against 30 volumes of a mixture of 1.65*M* NaCl and 0.004*M* phosphate at *p*H 7. Dialysis was started with the salt solution partly frozen to keep the temperature below 0°C until most of the alcohol had dialyzed out, and was completed at 2°C. The dialysis was repeated, and the solution (solvent density of 1.06) was then centrifuged at 97,000*g* for 18 hours. The pellet, which contains the α_2-macroglobulin, had a very high content of trypsin-binding protein, as measured by the determination of trypsin activity in the presence of an excess of soybean trypsin inhibitor. Hydrolysis of benzoylarginine-*p*-nitroanilide was followed at *p*H 7.7 in tris buffer by measuring the increase in absorbancy at 383 mμ (*1*, *9*). The substrate was prepared in a supersaturated aqueous solution, to avoid the necessity of including an organic solvent in the reaction mixture. Each assay tube contained 15 μg of trypsin (*10*), and this was allowed to react with the sample to be tested for 15 minutes before the addition of 20 μg of soybean trypsin inhibitor (*10*). Substrate was then added, and the extent of hydrolysis was determined after a fixed period of incubation, during which the rate of hydrolysis was constant. Values for the binding protein are given as the amount of trypsin which appears to be active in the presence of the inhibitor, and are calculated from the rate of hydrolysis relative to that obtained with 15 μg of trypsin, buffer, and substrate. Since the amounts of trypsin have not been corrected for the presence of any inactive trypsin in the Worthington trypsin (*11*), and since the combined trypsin appears to have only about 75 percent of the activity of free trypsin, the values for bound trypsin are apparent.

The lipid-poor material of the pellet from the fraction III–0 was further purified by gel filtration through Sephadex G-200 in 0.06*M* NaCl + 0.02*M* tris at *p*H 7.7. The elution patterns for material absorbing at 280 mμ and for trypsin-binding activity are shown in Fig. 1. The trypsin-binding activity is expressed as micrograms of combined trypsin per milliliter, and is obviously associated with the material of higher molecular weight. Fractions 60 to 80 were pooled for comparison with material prepared from plasma by a series of chromatographic steps which were designed to purify the trypsin-binding activity. Various preparations of the latter, which will be referred to as binding protein, had given ratios of apparent trypsin bound per milliliter to absorbancy at 280 mμ of 30 to 50. The material from Sephadex G-200, which we will refer to as III-0 macroglobulin, gave a value of 42 for this ratio. Approximately 1 percent solutions of

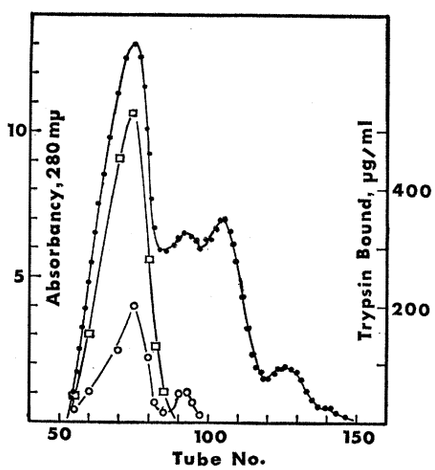

Fig. 1. Gel filtration of lipid-poor euglobulin from Cohn Fraction III-0 through Sephadex G-200 in a mixture of 0.06*M* NaCl and 0.02*M* tris, *p*H 7.7. Solid circles, absorbancy at 280 mμ; the scale is on the left. Open squares, trypsin-binding protein, and open circles, total trypsin inhibitor; results are expressed as apparent trypsin bound per milliliter of fraction; this scale is on the right.