Gill, Baxter and Moschella(7) used the indirect basophil degranulation technique to study serum from 100 patients with a history of sensitivity to penicillin and from 56 control subjects with no such history. They, too, found frequent degranulation in the serum controls.

In examining Shelley's published observations it is clear that his experiments were done with adequate biological controls. It is not clear whether a serum control was run with every individual test. From our data and those of Friedlaender and Friedlaender and Katz *et al* it is evident that a serum control is necessary for each individual serum tested. The difficulties of reading the slides are such that prejudice is readily introduced. Thus positive results can only be accepted if the slides have been read double blind.

*Summary and conclusions.* The "indirect" basophil degranulation technique was evaluated by replicate "blind" analyses of 8 sera. The results indicate that degranulation of basophils was a random phenomenon which was not influenced significantly by the presence or the absence of the antigen in the system or by the presumed presence or absence of antibody in the serum.

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## Nucleoside Phosphates in Liver of Mice During Experimental MHV-3 Virus Hepatitis.* (29651)

GIUSEPPE GIUSTI, BRUNO GALANTI, ANTONIO MANCINI† AND FERNANDO DE RITIS
(Introduced by A. B. Sabin)
*Clinica delle Malattie Infettive della Universita di Napoli, Italy*

Viral multiplication appears to be associated with the utilization of energy derived from oxidative phosphorylation. Anaerobiosis, reduction of oxygen consumption and dissociation of oxidative phosphorylation (obtained by different compounds devoid of virucidal activity), depresses the multiplication of certain viruses under different experimental conditions(1-5). However, in some strains of cells grown *in vitro* it has been possible to obtain active viral multiplication under anaerobic conditions(6). This is probably due to the high glycolytic activity typical of such cells grown *in vitro*. We have previously reported various metabolic modifications induced by viral multiplication in animals(7-10). This paper describes an investigation on nucleoside phosphates in the liver during experimental MHV-3 virus hepatitis in mice.

*Methods.* White mice, strain N.M.R.I., RECORDATI breed, weighing 12-16 g were fed with balanced synthetic diet "Altromin" of Altrogge Specialfutterwerk, Lage/Lippe, Germany. The MHV-3 virus (originally derived from the strain of Dr. Gledhill) was obtained from the ATCC. The following reagents were used: ATP-Test combination—"Enzymatische bestimmung von Adenosin-5'-triphosphorsäure"; ADP/AMP-Test combination—"Enzymatische bestimmung von Adenosin-5'-diphosphorsäure und Adenosin-5'-monophosphorsäure" of Biochemica Boehrin-

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† Research fellow of CNR.

ger, C. F. Boehringer and Sons, Gmbh. Mannheim. Perchloric acid, approximately 70% "pro analysi," E Merck AG Darmstadt.

Mice were inoculated intraabdominally with 10,000 $LD_{50}$ of MHV-3 virus, which was regularly virulent for the strain of mice used in these experiments. The $LD_{50}$ was calculated by the method of Reed and Muench. Nucleoside phosphates were determined on a perchloric extract of liver neutralized with triethanolamine-$KCO_3$ (TRA-$KCO_3$). To extract the nucleoside phosphates without hydrolysis, a slight modification of Carlson's method (personal communication to Streheler(11)) was adopted. Animals were sacrificed by decapitation. The livers were quickly removed and immersed in dry ice and petroleum ether, in this way bringing about the instantaneous freezing of the whole liver. The frozen livers were homogenized in a Potter homogenizer with 6% perchloric acid; final concentration was 10% (W/V). The homogenate was immediately centrifuged at 2,000 $\times$ *g* for 10 minutes and the supernatant fluid was neutralized with TRA-$KCO_3$. The potassium perchlorate precipitate was removed by centrifugation at 2,000 $\times$ *g* for 5 minutes and the supernatant fluid was used for the determinations. The nucleoside triphosphates were determined spectrophotometrically by the technique of Adam(12). By this method the limiting factor is the amount of triphosphate present during the reaction catalyzed by phosphokinase-3-glycerate.‡ The 1-3 diphosphoglycerate formed, stoichiometrically related to the amount of nucleoside triphosphate present, was measured spectrophotometrically by the addition of phosphoglyceraldehyde dehydrogenase and DNPH according to the following equations:

Nucleoside triphosphate + 3-phosphoglycerate PGK, $Mg^{++}$ → Nucleoside diphosphate + 1-3 diphosphoglycerate

1-3 diphosphoglycerate + DPNH + H GAPDH → Glyceraldehyde-3-phosphate + DPN + $PO_4$

Adam's method was used to determine the nucleoside diphosphates and adenosinemonophosphate(13). In the presence of an excess of phosphoenolpyruvate and crystalline pyruvate kinase, Nucleoside diphosphates represent the limiting factor in the synthesis of pyruvate.§ The pyruvate formed (stoichiometrically related to the nucleoside diphosphate present) was measured spectrophotometrically by the addition of lactic dehydrogenase and DPNH according to the following equations:

Nucleoside diphosphate + PEP PK,$Mg^{++}$, $K^+$ → Nucleoside triphosphate + pyruvate

Pyruvate + DPNH + H LDH → lactate + DPN

After nucleoside diphosphate determinations, adenosine monophosphate was measured in the same samples after conversion to adenosine diphosphate by the addition of crystalline myokinase.‖ The determinations were performed at 6, 12, 24, 36, 48 and 72 hours after inoculation of virus. Control animals, which were kept in identical environmental conditions, were used in all experiments. The animals were fasted 6 hours prior to sacrifice. The concentration of virus in the livers of infected mice was also determined at various intervals after inoculation.

*Results.* During the first 24 hours after inoculation, when first extensive viral multiplication is already in evidence, there was no significant change in concentration of nucleoside phosphates in the liver (see Table I). At 36 hours there appeared a statistically significant decrease in nucleoside triphosphate from 2.20 $\mu$moles/g in the controls to 1.77 $\mu$moles/g in the infected animals. Dur-

‡ ATP, ITP, GTP, UTP all react quantitatively in the same system. CTP does not react in measurable amounts. Since there is only a small difference in the reaction rates, differentiation of single nucleosides in a mixture is not possible(12).

§ADP, CDP, IDP, UDP, GDP all react quantitatively as phosphate acceptors, but at different rates (13).

‖ Muscle myokinase catalyzes only the transfer of phosphate radicals between adenylic nucleotides. We have used Boehringer myokinase, extracted from dog muscle.

TABLE I. Concentration of Nucleoside Phosphates in Livers of Mice at Various Times After Inoculation of 10,000 $LD_{50}$ of MHV-3 Virus in Relation to Level of Viral Multiplication.

| Hr after inoculation of virus | Titer of virus in liver* $LD_{50}$, $10^-$ | Nucleoside phosphates ($\mu$mol/g moist liver) † at indicated No. of hours after inoculation of virus | | | | | |
|---|---|---|---|---|---|---|---|
| | | Triphosphate | | Diphosphate | | Monophosphate‡ | |
| | | Mean ± S.D. | P | Mean ± S.D. | P | Mean ± S.D. | P |
| None (controls) | — | 2.20 ± .39 | — | 2.08 ± .22 | — | 1.16 ± .28 | — |
| 6 | 2.1 | 2.05 ± .84 | >.1 | 2.16 ± .33 | >.1 | 1.31 ± .12 | >.1 |
| 12 | 3.4 | 2.10 ± .29 | " | 2.11 ± .30 | " | 1.14 ± .17 | " |
| 24 | 6.4 | 2.64 ± .37 | " | 2.02 ± .20 | " | 1.01 ± .36 | " |
| 36 | — | 1.77 ± .31 | <.01 | 1.90 ± .12 | " | 1.19 ± .15 | " |
| 48 | 7.0 | 1.24 ± .55 | <.001 | 1.20 ± .51 | <.001 | .80 ± .29 | <.001 |
| 72 | 6.8 | 1.17 ± .61 | " | 1.03 ± .42 | " | .70 ± .15 | " |

* Each titration was performed on a pool of 5 livers.

† The livers of 60 uninoculated mice were used for obtaining the control values, and 10 mice were used at each indicated interval after inoculation of virus.

‡ Only adenosine monophosphate was measured in the assay for nucleoside monophosphates.

ing this phase of the infection the decrease was not accompanied by a similar decrease in nucleoside diphosphates and monophosphates. At 48 and 72 hours a still further decrease in the nucleoside triphosphates occurred, reaching 1.24 $\mu$moles/g and 1.17 $\mu$moles/g, and this was accompanied by a statistically significant decrease in the nucleoside diphosphates and monophosphates. Occasionally, during the course of infections the animals stopped gaining weight; there were, however, no statistically significant differences in total liver weight. Therefore the variations reported in Table I would also obtain if expressed as nucleoside content of total liver.

*Discussion.* It seems of special interest that the decrease in nucleoside triphosphates, first observed at 36 hours after inoculation when extensive viral multiplication had already occurred, was not accompanied by a parallel decrease in the other nucleoside phosphates. These data indicate that biochemical lesions of the nucleosides at high energy levels precede the damage associated with extensive hepatic necrosis in the later phase of infection. In the early stages of active viral multiplication between 12 and 24 hours after inoculation of virus there was no decrease in the nucleoside phosphates. These results are in agreement with the conclusions of others that the energy necessary for viral synthesis (with the exception, perhaps of infected cells cultured *in vitro*) is supplied by oxidative phosphorylation. For example, our data agree with that of De Ritis *et al*(14) and of Piazza(15), on the dissociation of oxidative phosphorylation and with that of Coltorti *et al*(16) on mitochondrial damage always found in the early phases of viral infection. Decrease in ATP content or in ATP synthesis has also been seen in liver removed by biopsy from patients with viral hepatitis(17,18). However, it is to be noted that the clinically apparent phase of the disease corresponds to a period relatively late in infection. During this late stage it may be assumed that viral multiplication has reached a low level or is almost finished. Such a situation is different from that of the experimental MHV-3 virus hepatitis studied by us in its earliest phases when viral multiplication has its maximum activity.

*Summary.* The concentration of nucleoside phosphates was determined in the liver of mice at various intervals after experimental infection with MHV-3 virus. During the first phase of active viral multiplication, occurring between 12 and 24 hours after inoculation of virus, there was no change in concentration of the nucleoside phosphates. A significant decrease only in nucleoside triphosphates was first found at 36 hours after inoculation when hepatic necrosis is only initial. This decrease became more marked at 48 and 72 hours at which time there also appeared a statistically significant decrease in nucleoside di- and mono-phosphates.

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## Fatty Acid Composition of Plaque and Tissue Lipids from Pigeons with Spontaneous Atherosclerosis.* (29652)

FRANKLIN YOUNG, CHARLES C. MIDDLETON AND HUGH B. LOFLAND, JR.
*Departments of Biochemistry and Laboratory Animal Medicine, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.*

The origin of lipids in the atherosclerotic plaque from aortas of man and animals remains obscure. Studies of the fatty acid composition of various tissues have been made in man(1-3), chicken(4,5), rabbit(6), rat, dog, goose, pig, and guinea pig(7). In most of the laboratory animal species, arterial lesions could be induced only after drastic alteration of diets(8-11). Clarkson *et al*(12) reported that the White Carneau pigeon, even when fed a diet of grains, develops atherosclerosis having many of the morphological characteristics of the human disease. The study reported here was conducted to determine the fatty acid composition of plaques and other tissues of these pigeons when the birds were fed a "natural" diet.

*Materials and methods.* Sixty White Carneau pigeons, 5-8 years of age, were obtained from the Palmetto Pigeon Plant, Sumter, S. C. The pigeons were divided into 6 groups of 10 each. They had been fed corn, wheat, milo, and peas *ad libitum*. At necropsy all of the birds had grossly visible atherosclerotic lesions of the aorta. The tissues collected and pooled according to groups were: livers, sera, excised atherosclerotic plaques from aortas, and non-diseased portions of aortas. Special care was taken to remove all of the extraneous tissue from the adventitia of the aortas prior to the excision of lesions. Since the lesions almost always occurred at the distal segment of the thoracic aorta near the coeliac bifurcation(12), the segment from the aortic arch to a few millimeters proximal to the diseased area was taken as the non-diseased aorta. For serum and liver, duplicate samples were taken from each pool.

Lipids were extracted from the tissues according to Folch *et al*(13) with modifications. All solvents except ethanol and diethyl ether were redistilled. The tissue was homogenized in chloroform:methanol mixture, 2:1 (v/v), in the proportion of at least 20 ml of mixture per gram of tissue. For serum, 5 ml of the sample were slowly added to 100 ml chloroform:methanol mixture with con-

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