ELECTRON MICROSCOPY OF THYMONUCLEIC ACID

by

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An electron microscope study of thymonucleic acid has been undertaken in the hope of obtaining further data on the structural periodicity of this complex long-chain compound.

The specimens studied were pure samples of calf thymus desoxyribose nucleic acid prepared by D. L. WOODHOUSE, using a modification of the MIRSKY process. Analysis showed the following composition: phosphorus 8.5%, nitrogen 15.1%, total purines and pyrimidines 37%, protein (by SAKAGUCHI reaction) 1.4%. The DISCHE colour value compared with phosphorus value indicated absence of ribose nucleic acid.

The experiments have been carried out with a Metropolitan-Vickers electron microscope operated at 75,000 volts, the micrographs being recorded on Kodak plates L 15. The magnifications have been determined with the help of a calibrated aperture in a selected grid, which was compared, for each given value of lens current, with the edge of the final image field whose dimensions are accurately known. The value of the magnification given with each figure is assumed to be known with no more than $\pm$ 10% error.

Observations were made for various initial states of aggregation in water, from a solution (0.25 to 0.50%) to a gel-like consistency, and the appearances recorded vary greatly with the quantity of water present initially and with the technique employed in the preparation of the specimens. The samples were shadowed with gold-palladium at an angle of 30°.

When the aqueous solution is placed directly as droplets and dried on the collodion-covered grid of the microscope, the substance breaks up into spherical units distributed mostly at random but of even size. Their dimension is difficult to evaluate with accuracy owing to the thickness of the shadowing between the particles. By measuring, however, at right angles to the direction of shadowing, it can be computed that their diameter is of the order of 160 A. It must, of course, be remembered that the desiccation of the sample has shrunk the groups or chains of molecules under observation.

Other samples have been prepared from the dry fibrous material which has been spread on a collodion-covered slide with a fine sable brush loaded with water. The gel spread in this manner shows a definite structure. To eliminate the surface effect due to the spreading of the material on the slide and the pebbly appearance of the collodion film itself, micrographs have also been recorded of gels spread directly across the grids, without the support of collodion (Fig. 2). The spherical units are organised in fibrils which form a network, the appearance being that of an irregular honeycomb. The number of spherical elements constituting one side of the holes in the mesh can be counted and does not seem to be constant (2 to 4). In some parts of the micrographs the mesh disappears and the structure reverts to linear arrays or to the random distribution observed previously (Fig. 1). Some holes are hexagonal in shape, which is of interest in view of the observations of J. A. V. BUTLER (X-ray diffraction experiments, British Empire Cancer Campaign Report 1950, p. 301). Within the net-work, streaks

(Text continued p. 10).

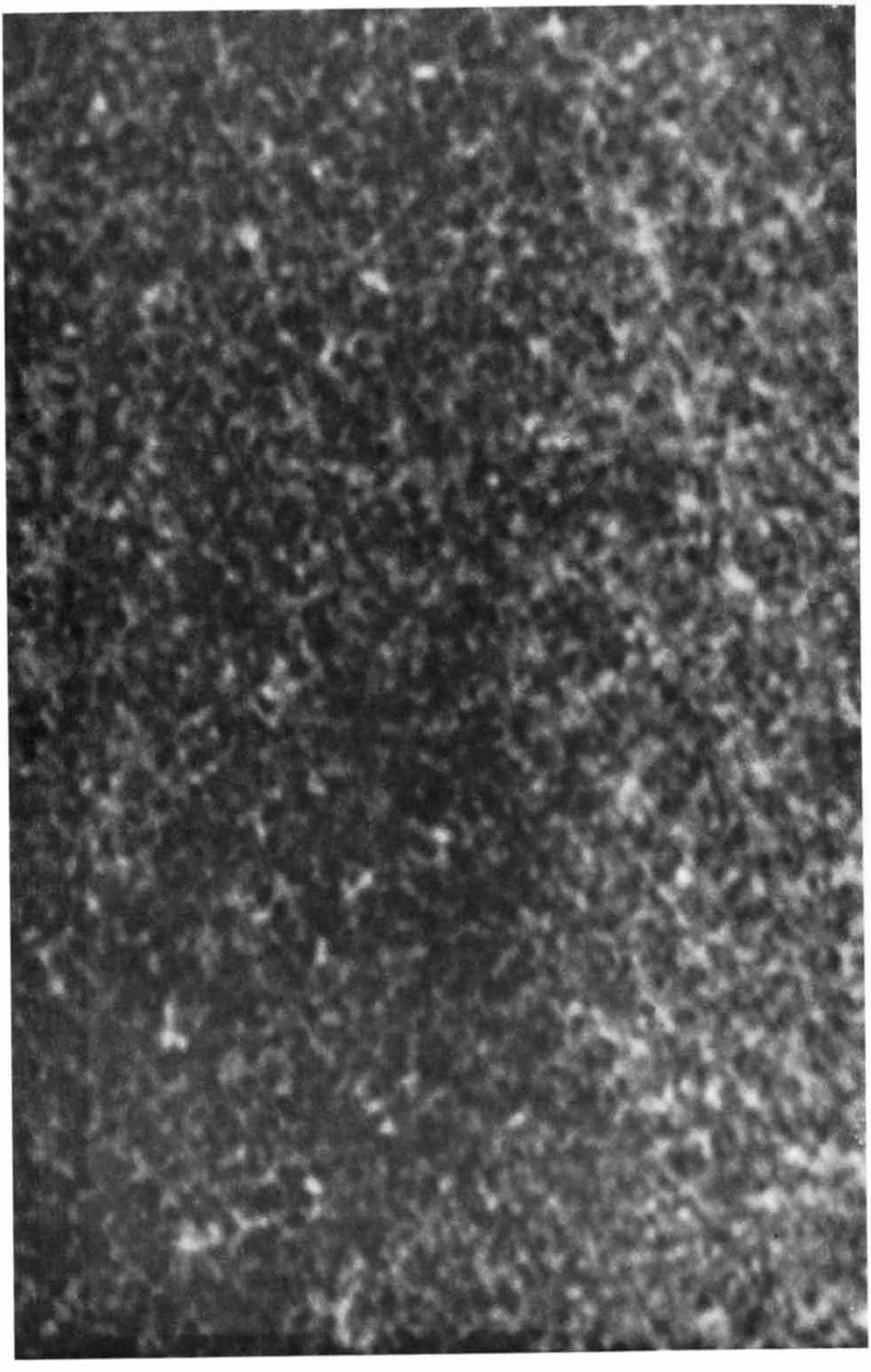

Fig. 1. Thymonucleic acid, dried on microscope grid, gold-palladium shadowed, × 100,000

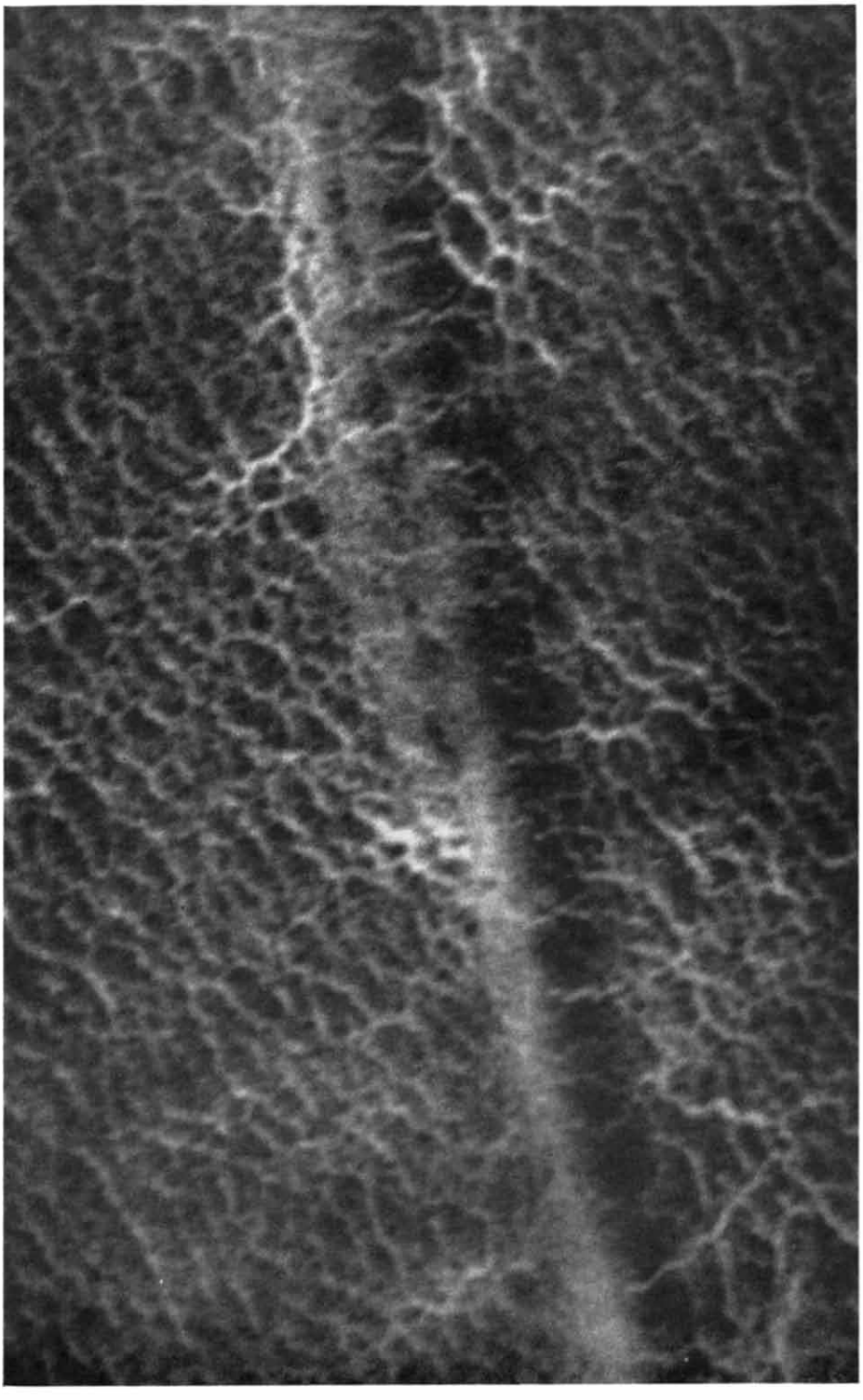

Fig. 2. Thymonucleic acid film, unsupported by collodion, gold-palladium shadowed, × 120,000

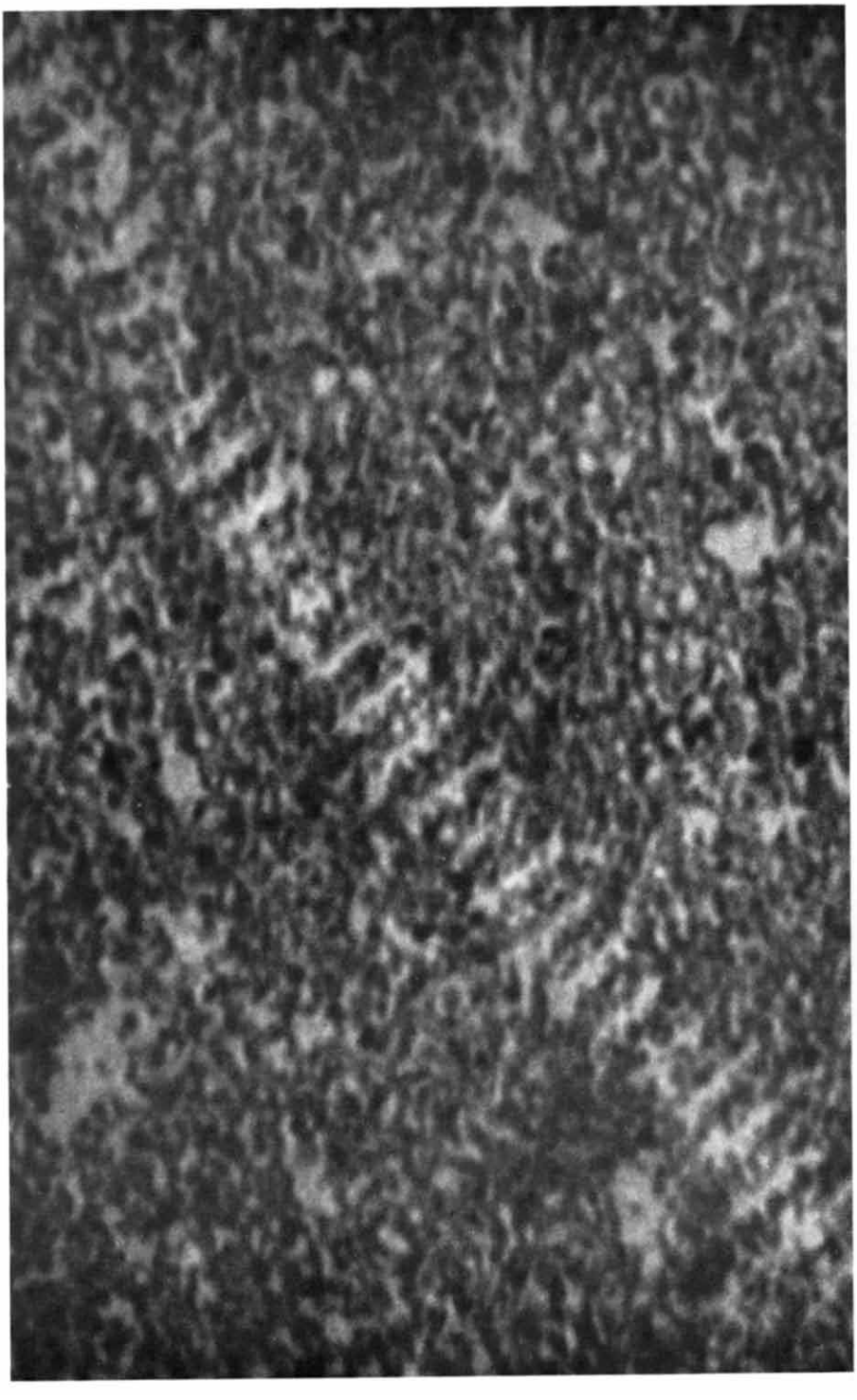

Fig. 3. Thymonucleic acid gel spread on collodium, gold-palladium shadowed, × 100,000

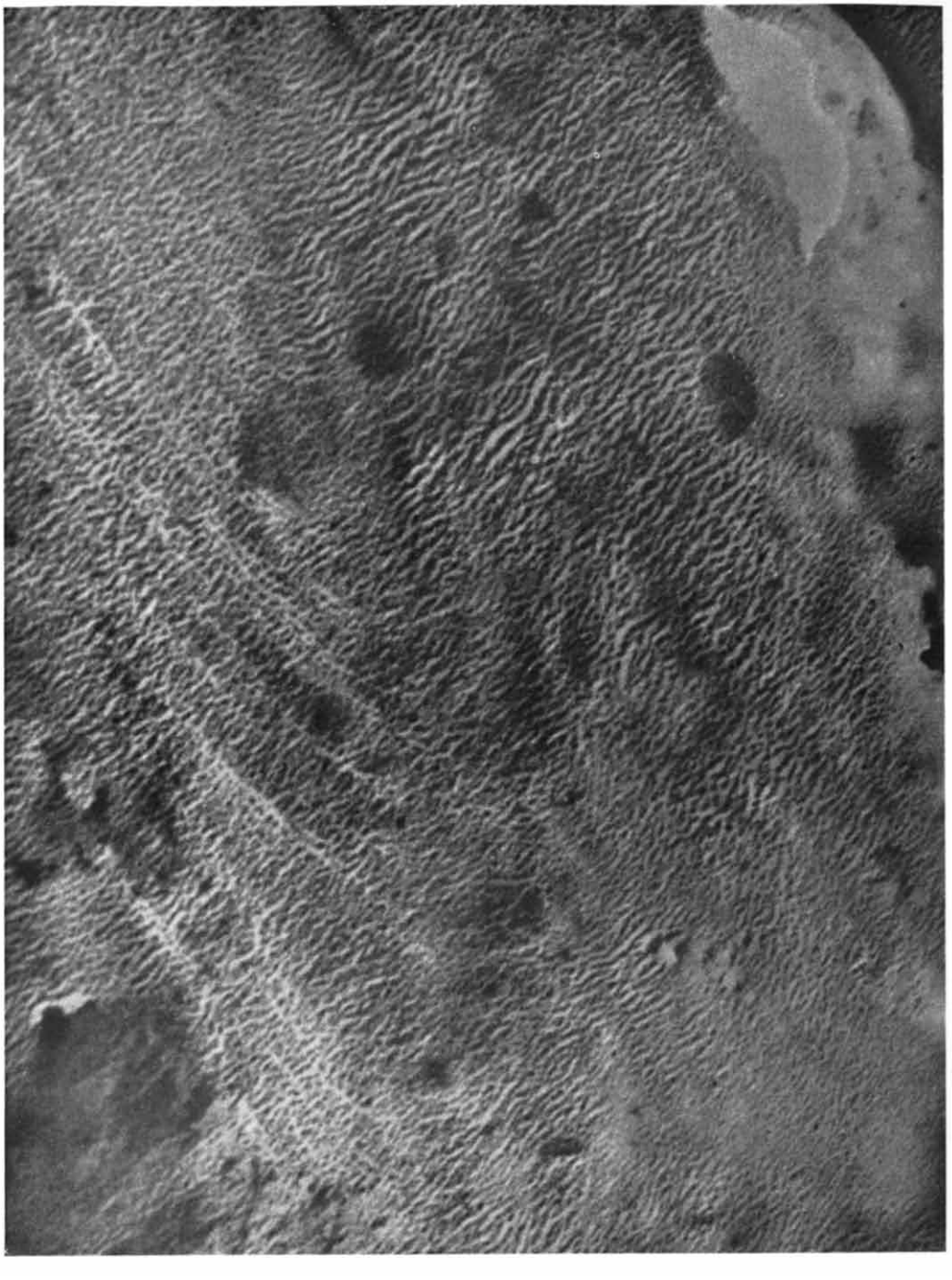

Fig. 4. Thymonucleic acid (second specimen) unsupported by collodion, gold-palladium shadowed, × 35,000

of the material are frequently visible, as in Fig. 2 and 3, giving the impression of fibres blending with the mesh and presenting a ribbed structure with a constant spacing, circa 800 A, between the bands, alongside the axis of the fibre.

In the samples prepared by spreading the dry fibrous material on collodion-covered slides with a brush, if excess of water is avoided well-defined fibres are observed; when they are thin enough for resolution, they show a banded structure similar to that observed within the net-work of the gel and with the same spacing. However, as there is a possibility of a small number of connective tissue fibres remaining, in spite of repeated separations and centrifugations, further work is now being carried out on desoxyribose nucleic acids obtained from other sources and on thymonucleic acid treated by desoxyribonuclease.

Another sample of thymonucleic acid, which has been submitted to repeated Sevag extractions in the Waring blendor, has also been studied and micrographs of the gel unsupported by collodion have been recorded (Fig. 4). A honeycomb type of structure is observed here also, but the spherical components appear to be smaller.

I am indebted to Professor SAYERS of the Physics Department, Birmingham University, for facilities, and I wish to acknowledge the technical help of Mr C. C. NEWTON. This work was done under a grant from the British Empire Cancer Campaign, Birmingham Branch.

SUMMARY

Samples of calf thymus nucleic acid prepared by Mirsky's method have been examined in the electron microscope. Different appearances have been recorded according to the initial state of aggregation of the aqueous systems studied, which was varied from a solution (0.25%) to a gel.

When droplets of the solution are dried directly on the grids, the micrographs show that the material splits up in spherical elements, distributed at random, but of even size (diameter circa 160 A).

The films formed by spreading the gel onto the grid without support of collodion show a definite structure. The spherical units are organised in fibrils which form a network. The holes in the mesh are of different sizes but are often hexagonal in shape.

Amongst the network, streaks of the material, looking like fibres, are frequently observed. They present a banded structure, with a constant spacing at right angles to their axis (circa 800 A), and they blend with the network.

RÉSUMÉ

Nous avons examiné au microscope électronique des spécimens d'un acide désoxyribose-nucléique préparé par la méthode de Mirsky. Différentes techniques ont été utilisées pour placer la substance sur les grilles porte-objet.

Pour les specimens les plus dilués, placés en gouttelettes et desséchés sur collodion, on observe une séparation de la substance en éléments sphériques sans orientation ou organisation apparente, mais d'un diamètre constant de l'ordre de 160 A.

Il est possible, à partir du gel, de fixer sur les grilles un film sans support de collodion et les micrographies obtenues révèlent une structure fibrillaire bien définie. Les éléments sphériques constituent, en s'alignant, des fibrilles qui forment un réseau ou nid d'abeilles. Les mailles de ce réseau sont inégales, mais présentent souvent une forme hexagonale.

Sur nombre de micrographies, on observe des trainées de matière ayant l'apparence de fibres à bandes régulières, dont les éléments sont identiques aux particules du réseau et se fondent avec elles. L'intervalle entre ces bandes transversales est très régulier et est de l'ordre de 800 A.

ZUSAMMENFASSUNG

Proben von Thymusnucleinsäure, nach Mirsky's Method hergestellt, wurden unter dem Elektronenmikroskop untersucht. Verschiedene Erscheinungen wurden verzeichnet, entsprechend dem Anfangsstadium des Aggregats, das von einer Lösung (0.25–0.50%) bis zu einem Gel variierte.

Wenn Tröpchen der Lösung direkt auf dem Gitter des Mikroskops getrocknet werden, zeigen die Mikrographen, dass das Material sich in kugelförmige Elemente aufspaltet, die ganz unregelmässig verteilt, aber von gleichmässigem Durchmesser sind (160 A).

Die durch Ausbreiten des Gels über dem Gitter ohne Kollodionbasis gebildeten Deckhäutchen zeigen ganz deutliche Struktur. Die kugeligen Körperchen sind faserig gestreift, so dass ein Netz entsteht. Die Netzlöcher sind von verschiedener Grösse, aber oft sechseckig.

Im Netz lassen sich Streifen des Stoffs beobachten, die wie Fasern aussehen. Sie stellen eine Bandformung in regelmässigen Abstanden (circa 800 A) dar und verschmelzen mit dem Netz.

Received July 8th, 1952