cedure[14]. Variable times of incubation were used with all the techniques to evaluate the relative enzymatic activity.

There was a similar and very high enzymatic response for all enzymes in all types of multinucleated giants cells studied. Succinic-dehydrogenase and phosphamidase reacted more specifically, i.e. the giant cells gave a frank response against the neighbouring cells that showed weak reaction only. Acid phosphatase reacted with greater intensity in the giant cells and, though to a lesser degree, there was also activity of the osteoblastic elements in the bone tissue and neighbouring histiocytic cells of the granuloma. β-Glucuronidase appeared in high proportion in the giant cells, though it was also seen in the osteoblastic elements and to a lesser degree in the histiocytic reticular cells. In the experiments carried out with parathyroid hormone, an increase of these enzymes was found in bone tissue, related to the increase of the osteoclastic type of giant cell. There was also a marked reaction of acid phosphatase in the fibroblastic near the trabeculae.

Our results show that foreign body giant cells are rich in enzymes. This would indicate a very intense metabolic activity probably related to the absorption mechanism. The fact that the response in this group of enzymes for osteoclasts, chondroclasts and foreign body giant cells was alike, induced us to assume a similar enzymatic pattern for the absorption mechanism of different substances.

Zusammenfassung. Intensive enzymatische Aktivität (saure Phosphatase, Succinodehydrogenase, Phosphamidase und Glukuronidase) wurde in Osteoklasten-Fremdkörperriesenzellen und vielkernigen Riesenzellen in einer grossen Zahl von pathologischen Knochenprozessen festgestellt. Die Bedeutung dieser Befunde für den Mechanismus der Absorption wird diskutiert.

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Registro Latinoamericano de Patología Osea and Comisión Nacional de Energía Atómica, Buenos Aires (Argentina), March 2, 1962.

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Hyperoxia and Formation of Chromolipoid Pigments

Chromolipoid pigments (also called lipofuscin, wear and tear pigment, ceroid, hemofuscin) occur widely in various cells of mammals. Their amount is known to increase with age and also in various situations in which oxidation of unsaturated lipids is enhanced locally or in the whole body[1]. It is known that the amount of these pigments is increased in animals suffering from avitaminosis E[2] (i.e. in the absence of the anti-oxidant activity of this vitamin), in iron overload[3] and in hemorrhages in lipid-containing tissue[4] (i.e. in the presence of iron compounds which catalyze oxidation); the pigmentation also occurs in cirrhotic livers[5]. These data indicate, as has been previously stated[1], that the formation of these pigments depends: (a) on the local presence of unsaturated lipids, and (b) on the presence of conditions favouring oxidation. Chemical studies[6–9], as well as histochemical studies and experiments of producing similar pigments *in vitro*[6,10–13], have shown that the pigments are the products of peroxidation and polymerization of unsaturated lipids.

It appeared reasonable to assume that repeated exposures of animals to high O_2 tension might also cause the formation of lipid peroxides and of chromolipoid pigmentation. The present report deals with experiments performed to test this notion.

White rats were exposed 6 days a week to high O_2 tensions. The container in which the rats were exposed to O_2 was rinsed three times with pure oxygen by raising the pressure to 3.5 atm and then letting the gas flow out. The gas mixture in the container was calculated to contain 98% O_2. The animals were exposed for 20–30 min to O_2 at 3.5 atm during the first week. As from the second week the pressure was raised to 4 atm and as from the middle of the third week the time of exposure was raised to 40 min.

Two exposed animals and two controls fed a similar diet were sacrificed weekly with ether. The last animals were killed 60 days after the first exposure to O_2. Pieces of brain, cerebellum, heart, liver, spleen, kidney and ileum were fixed in formalin and embedded in paraffin. Seven microns thick sections were stained with hematoxylin, Sudan black B, Ziehl-Nielsen and the P.A.S. procedures. Another section was mounted unstained in glycerin for examination with ultra-violet light for auto-fluorescence.

Pieces of the brains of animals killed in the last two weeks were homogenized and examined by the thiobarbituric acid method for the amount of lipid peroxides in them.

Study of sections stained with Sudan black, Ziehl-Nielsen and the P.A.S. procedures indicated a slightly higher content of stainable material in the O_2 exposed groups than in the non-exposed as from about the fourth week of O_2 exposure. The stainable material was found mainly in adventitial histiocytes in the various organs, but also in histiocytes of the spleen and liver and in neurones. The results were however erratic with marked individual variations.

Less equivocal changes were found in sections examined for autofluorescence with ultra-violet light. More material which emitted bright yellow fluorescence was found in the O_2-treated than in the non-treated animals as from about the fourth week. The yellow fluorescence was mainly concentrated in the capillary walls and, although it was present in all animals, there were marked differences in intensity between groups.

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On the two occasions in which the amounts of thiobarbituric acid reactive materials were estimated in the brain, the amount of lipid peroxides in the O_2-treated group was found to be almost twice that found in the animals not treated with O_2.

It is possible that the total length of exposure to O_2 (total of 30–40 h in the animals which were kept alive for the longest period) was not long enough to produce more marked changes. In the presently described experiment, highest O_2 tensions probably prevailed in the blood stream and in the walls of blood vessels. Also in the autopsy described by LUND[14] most chromolipoid deposition in the brain was perivascular. Perivascular pigmentation was also observed in the central nervous system of various vitamin E deficient animals in addition to neuronal pigmentation[15,16].

It appears therefore that the blood vessel walls and specially those of the central nervous system play an important role in the determination of lesions by hyperoxia. The possibility that unsaturated phospholipids and other unsaturated lipids are the primary target of hyperoxia appears to be logical and is being further studied[17].

Zusammenfassung. Ratten, die wiederholt hohen O_2-Drucken ausgesetzt wurden, zeigten nach 4 Wochen erhöhte chromolipoide Pigmentation. Die Pigmentation blieb relativ gering und lag hauptsächlich in der Gefässwand und deren Umgebung bzw. den Stellen höchsten O_2-Druckes.

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Potassium Levels in Human Semen with Reference to Sperm Motility

Mammalian seminal plasma is known to contain sodium, potassium, calcium, magnesium, and traces of heavy metals (MANN[1]). The effect of these metals and related ions on the metabolism and motility of the mammalian spermatozoa has been of great interest to workers concerned with the metabolism of spermatozoa. It was observed by WALES and WHITE[2,3] that, in the case of dog and fowl, low concentrations of potassium were necessary for optimal metabolism of spermatozoa, whereas high concentrations were found to be detrimental. CRAGLE and SALISBURY[4] found that oxygen uptake and glycolysis of bull spermatozoa were reversibly inhibited by potassium concentrations of 200 to 280 mg/100 ml. The high potassium content of semen also had a deleterious effect on motility. It has already been reported by SHETH and RAO[5] that 0.05 *M* KCl inhibited fructolysis completely. These results indicated that more than optimal concentration of potassium may also adversely affect motility of spermatozoa. It was therefore of interest to determine the potassium content of human semen with spermatozoa with different percentage motility. Preliminary results are presented in this communication which give an account of the potassium levels in 120 normal and subnormal semen samples.

Semen samples used in these studies were obtained from fertile donors and from men referred for infertility by the K.E.M. Hospital, Parel, Bombay. Soon after liquefaction of the semen (about $^1/_2$ h after ejaculation) the spermatozoal count and percentage motility were determined, using standard methods. Semen was centrifugated for 30 min at 2000 r.p.m. to separate spermatozoa and seminal plasma. Potassium levels in semen and seminal plasma were determined by the method of KING[6].

Results given in the Table show that potassium content of seminal plasma accounted for almost all the total potassium content in whole semen. The potassium values of seminal plasma were found to be a little higher than those of the respective semen sample. This may be due to the difference in volume brought about by the displacement of seminal plasma by the different volumes of spermatozoa in the individual semen samples.

These results are in contrast to those reported by CRAGLE, SALISBURY, and VAN DEMARK[7], who reported a higher concentration of potassium in bovine spermatozoa as compared to bovine seminal plasma. In the work reported here only seminal plasma was used to determine the levels of potassium since potassium was not present in spermatozoa in any significant amounts. In all, 120 semen samples were used to measure the potassium levels in the respective seminal plasma. Semen samples were arbitrarily divided into groups according to the percentage of motile spermatozoa present. Those samples in which more than 30% of spermatozoa were motile were considered to have good motility, while those with 30% or less than 30% motile spermatozoa were considered to be of poor motility. The mean potassium level in good motility samples was found to be 126 mg/100 ml (S.E. $\pm$ 1.2) and was compa-

Potassium levels of semen and seminal plasma

Sample number	Count/ml in millions	Percentage motility	Potassium values mg/100 ml Semen	Seminal plasma
1	40	50	148	152
2	300	40	140	148
3	80	30	196	202
4	60	50	138	140
5	200	40	128	132

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