Day-12 and day-13 hamster embryo response to maternal, i.p. injection of deionized, distilled water or copper citrate (2.7 mg/kg) administered on the morning of the 8th day of gestation

Group	Number of hamsters	Number of implantations	Number (%) of embryos live at sacrifice	live embryos with edema	live embryos with heart defects	Percent of edematous embryos with heart defects	Number of hearts examined microscopically
Deionized, distilled water	12	150	145 (97)	0 (0)	0 (0)	0	50
Copper-treated	17	215	144 (67)	21 (15)	7 (5)	33	34

vessels are traced into the heart however, the aorta comes to lie dorsal to the pulmonary trunk and connects with the left ventricular outflow tract. The ventricular septum is usually complete by day 12 and is well-developed by day 13. These features of cardiac development follow a different course in the affected embryos. The most striking abnormality is that the pulmonary trunk is remarkably narrow throughout its extent (figure). The pulmonary arteries are noticeably hypoplastic also. The aorta does not course behind the pulmonary trunk in the normal manner but comes to lie alongside this outflow tract. The result of this malpositioning is that the aorta and pulmonary trunk both arise from the right ventricle. In addition, the interventricular septum is deficient in these cases. This pattern of cardiac maldevelopment has been classified as pulmonary hypoplasia and double-outlet right ventricle with an associated ventricular septal defect.

The pathognomonic feature of this syndrome, which was present in the copper-induced cases, is that the aorta and pulmonary trunk both arise completely from the right ventricle[7]. A ventricular septal defect is an important adaptive component of the double-outlet right ventricle syndrome because it provides the only means of exit for the oxygenated blood in the left ventricle. A ventricular septal defect was found in all the experimentally-induced cases and is a consistent finding in human cases with this cardiac lesion[8]. Associated defects in the pulmonary trunk are also thought to have survival value. Pulmonary arterial stenosis is a common but non-essential component of this syndrome in humans[8], and it is of interest that pulmonary hypoplasia was present in all of the experimentally-induced cases in hamsters. Since the right ventricle becomes the systemic ventricular chamber in cases of double-outlet right ventricle, Taussig[9] suggested that a narrowing in the pulmonary trunk may protect the lungs from undue pressure.

The effects of copper on heart development are of considerable interest because animal models for the investigation of abnormal cardiogenesis are rare. A comprehensive study[10] in Keeshond dogs suggests that the occurrence of ventricular outflow tract lesions are under a genetic influence. The induction of a specific array of defects in the hamster heart by copper citrate renders this system a useful experimental model. Observation of embryonic hamster hearts following copper exposure at successively earlier stages, may reveal a sequence of events in the pathogenesis of double-outlet right ventricle.

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Octopus chromatophores accumulate nickel

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Summary. Microprobe analysis of the pigment granules in the superimposed chromatophore layers of *Octopus vulgaris* reveal rising amounts of calcium, nickel and sulfur as the size and electron-density of the granules increases. The colours, associated with these increases, shift from yellow/orange to red and finally black.

Octopuses and cuttlefish - like all other modern cephalopod molluscs - have a dense array of coloured spots (chromatophores) in the surface layers of their skin. The spot matrix is used by the nervous system to display a range of patterns and colour changes unmatched by any other kind of animal. As it is not known what particular pigments are responsible for the individual hues - yellow, orange, red, dark brown or black - that are revealed when the chromatophores are spread, we have looked at them under the electronmicroscope and used the opportunity of microprobe analysis to throw some light on their chemical nature.

The members of the chromatophore array lie in staggered positions relative to each other and in successive layers of

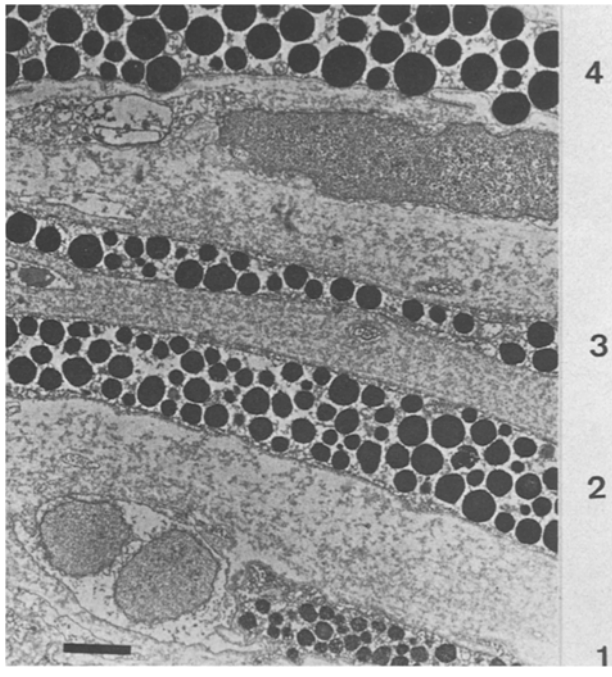

Fig. 1. Cross section through dorsal mantle skin of an octopus fixed in cacodylate-buffered osmic acid (1180 mosmol). Scale line 1 µm. Chromatophores lie at 4 different levels (1–4) in the dermis and contain pigment granules increasing in average size and electron density from below (1) upwards towards the epidermis. The mean diameters and standard deviations of the granules in the 4 levels are: *1* 272±144 nm, *2* 400±270 nm, *3* 406±314 nm and *4* 538±276 nm. (Values based on measures of 100 sectioned granules in each of 4 chromatophores. Section thickness about 100 nm)

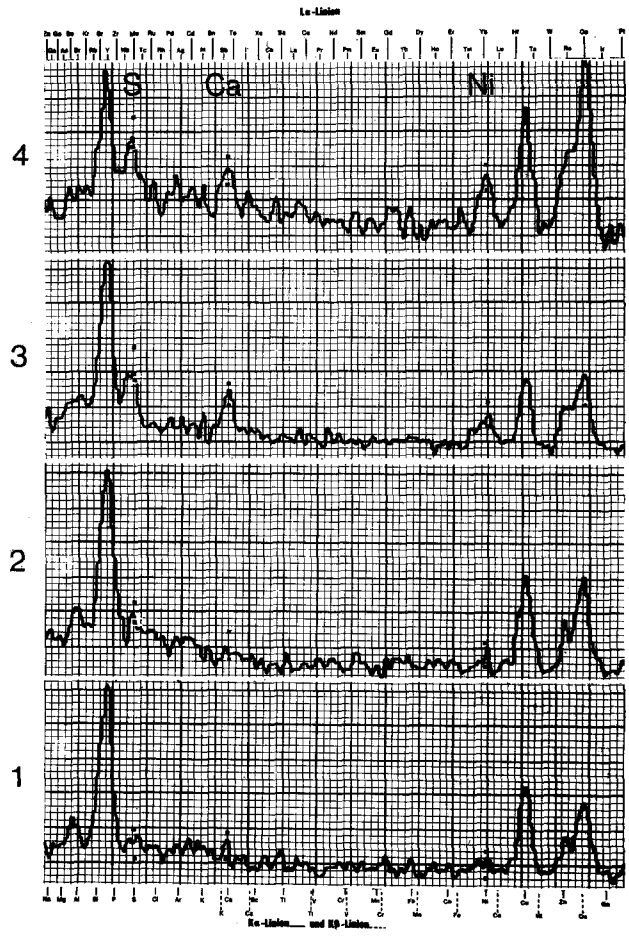

Fig. 2. X-ray emission spectra of single pigment granules in 4 different chromatophores. To exclude errors related to granule size, granules of about 300 nm (i.E. half the beam diameter) were analyzed. Copper and osmium peaks (right) originate respectively from the grid and the tissue fixative. Sulfur, calcium and nickel lines have peaks (indiscernible in 1) that increase in height from 2 to 4. The marks beside these lines indicate the range observed in 6–16 granules analyzed from chromatophores of 3 different animals. (Spectra recorded by a Kontron processing unit after 2 min of irradiation at 40 µA and 40 kV with a beam diameter of 0.6 µm using a Link EDX-system attached to a Zeiss EM 10.)

the dermis. Seen in life, the lightest (pale yellow) are the deepest and the darkest (black) are the most superficial. Figure 1 shows the appearance of the pigment granules in chromatophores at 4 levels normal to the surface of the skin of a common Mediterranean octopus, *Octopus vulgaris*. The granules of the lowest chromatophore (1) are small, show internal structure and have relatively low electron-density, while those of the highest (black) chromatophores (4) are large and uniformly opaque. The intervening chromatophores (2 and 3) contain granules of intermediate size and density.

X-ray emission spectra of individual granules (figure 2) show that the black ones (4) have peaks corresponding to the nickel and sulfur lines, but the lightest (yellow) ones do not, while granules in the intermediate layers (2 and 3) have peaks of intermediate height. Other electron-dense structures in this material (e.g. lysosomes, collagen, iridosomes) give no nickel peak.

The main candidates for the pigments in cephalopod chromatophores are ommochromes and melanins. Fox and Crane's early report[2] of melanin in extracts of whole skin from an octopus does not seem to have been confirmed, but ommines have now been repeatedly identified in extracts of cuttlefish skin[3], lately 3 ommochromes (distinguishable by their hue, solubility and redox performances) being tentatively equated with the yellow, orange/red and brown chromatophores of *Sepia officinalis*[4]. Ommines are synthesqed from tryptophan and contain sulfur as constituent from the amino-acid methionine[5]. If the sulfur revealed by the X-ray emission spectra is in the pigment molecule, then probably the nickel is also, as both peaks rise at the same rate.

Nickel is present in seawater (mostly as the hydrated Ni^{2+}) in concentrations between 0.05 and 0.5 µg/l[6]. It accumulates in the zooplankton to 2–8 µg/g dry weight[9] and may reach octopus chromatophores through the animals' diet, crustaceans and fish. Like copper, which is well-known as a constituent of the blood pigment hemocyanin, it may be an essential trace element for cephalopod molluscs. The divalent ion readily combines with sulfur giving a stable black precipitate (Ni (OH) S) in air. In common with the other transition elements, it forms coloured compounds, the square-planar ligands with organic nitrogen being frequently red, yellow or brown[7].

Whatever the chemistry of the nickel present in octopus chromatophores, we believe that the increases in the amount of the element and of sulfur that go with increasing density and size of the pigment granules in successive layers of the skin are all evidence that the orange, red-brown and black chromatophores containing these granules are to be regarded as members of a continuous series caught at successive stages of differentiation. We know that chroma-

tophores darken with age[8,9], the black ones developing from brown and the red ones from orange, etc., though we also know, on physiological grounds, that the different members of the coloured series are separately innervated and make distinct contributions to patterning at any one moment[10,11].

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Studies on the activity of phorbol myrystate acetate on the human polymorphonuclear leukocytes

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Summary. Phorbol myrystate acetate (PMA) activates nitroblue tetrazolium reduction in human polymorphs. The activation is inhibited by dibutyryl cyclic AMP, theophylline and phenylbutazone, but is not influenced by hydrocortisone in vitro, nor is it inhibited by leukocytes from patients treated with prednisone. Peptide analogues of Tuftsin also had no effect on this stimulatory activity. We conclude that the action of PMA on the nitroblue tetrazolium reduction is mediated through cyclic nucleotides.

Recently it was shown that phorbol myrystate acetate (PMA), the active substance of croton oil, when added to polymorphonuclear (PMN) leukocytes stimulates events occurring during phagocytosis of particles, i.e. the increase of oxygen consumption, hexose monophosphate shunt (MMP) activity and reduction of nitrous blue tetrazolium (NBT)[1,2]. The mechanism of action of PMA has not yet been explained, but there are some very important observations[1] prompting further studies.

We have previously reported that dibutyryl cyclic AMP and related substances decrease the NBT reduction by PMN leukocytes, while cGMP and related substances increase this reaction[3]. We have also demonstrated that when PMN leukocytes are preincubated with tuftsin (phagocytosis-stimulating peptide) analogs, the ability of the cells to reduce NBT is inhibited[4]. These findings and the importance of the model of PMA-stimulated intracellular changes led us to investigate the influence of cAMP, theophylline, cGMP, tuftsin and its analogs, hydrocortisone and phenylbutazone on the stimulatory effect of PMA in the system of quantitative NBT reduction test[5].

Material and methods. Samples of 20 ml venous blood from healthy young medical students were collected in 30 ml disposable plastic tubes containing 50 units heparin (thromboliquin) per 1 ml. 5 ml of 6% dextran solution (Fluka AG) were added to each tube and the mixture was allowed to settle for 1 h at room temperature.

The next steps were essentially as described by Baehner and Mathan[5] with the following changes: To each test tube containing 2.5×10^6 leukocytes in 0.1 ml, we added the following substances (instead of latex particles):

1. 20 µg endotoxin (B_4 lipopolysacharride, Difco, Detroit);
2. 1, 2, 5 ng phorbol myrystate acetate (12-O-tetradecanoyl phorbol-13-acetate, Cons. Midland Corp. Brewster N.Y.;
3. 1 µg or 10 µg of tuftsin (phagocytosis stimulating peptide L-Thr-L-Lys-L-Prol-Arg);
4. tuftsin analogs: Des^4-threonyl-tuftsin 0.1 µg - alanyl^4 tuftsin 0.1 µg (synthesized by us);
5. methylene blue $0.22 \times 10 \times 10^{-3}$ M;
6. hydrocortisone 100 ng, 200 ng, 500 ng;
7. theophylline (Sigma) 10^{-5} M;
8. carbacholamine 10^{-5} M (Sigma);
9. cAMP dibutyryl adenosine 3′5′cyclic monophosphoric acid 5×10^{-6} M (Sigma), cGMP Guanosine 3′5′cyclic monophosphoric acid (Sigma) 3×10^{-3} M;
10. phenylbutazone (Sigma) 5×10^{-3} M.

In addition, we studied the effect of PMA and endotoxin on PMN leukocytes obtained from 4 children treated by prednisone 2.5 mg/kg for 10 days at least (rheumatic fever (2), dermatomyositis (1) and nephrotic syndrome (1). After addition of 0.2 ml of 0.1% NBT (sigma), the mixtures were incubated for 30 min at 37 °C. The reaction was stopped by addition of 10 ml 0.5 HCl and centrifuged. The reduced NBT was extracted from the cells once in 3 ml pyridine (BDH) under a boiling water bath. Then the colour intensity was measured spectrophotometrically (Zeiss PM20DL) at 515 nm.

In the 2nd experiment we preincubated the leukocytes (handled as in the 1st experiment) separately with the following substances: Des-Thr tuftsin, Ala Tu, phenylbutazone, theophylline, dibutynyl cAMP, cGMP, carbachol, cAMP′-theophylline, carbachol′-cGMP, in the same concentrations as in the 1st experiment; then to each test-tube we added endotoxin or phorbol myrystate acetate and NBT suspension and continued as previously described.

The results of each test were expressed as ΔA per 2.5×10^6 leukocytes per 30 min, calculated as the difference in optical density between each reaction tube ('stimulated') and a blank.

Results. Figure 1 summarizes the principal data obtained from our experiments. PMA has a marked stimulatory