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Tumour Formation in Platyfish-Swordtail Hybrids as a Problem of Gene Regulation*

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1. *General aspects of genetically fixed tumour formation*

Tumour formation, not caused by external stimulations but only based on the genetic constitution of an organism, is often found in plants and animals[1]. Nevertheless, only 3 objects have been studied in detail with regard to tumour formation: *Drosophila melanogaster*[1,2], certain hybrids in the genus *Nicotiana*[1,3], and certain hybrids of viviparous cyprinodent fishes (*Poeciliidae*)[1,4,5]. In this paper will be discussed only the formation of melanomas in a group of the third object, in platyfish-swordtail hybrids. Since those melanomas originate from colour spots, it is necessary first to describe the genetic make-up of a typical spot pattern.

2. *The genetic dependence of the spot pattern*

Wild species of the genus *Platypoecilus*, known to laymen as the original forms of the domesticated platyfish, have different patterns of melanin-containing spots or stripes which are almost constant from generation to generation. Thus the pattern of these fish is inheritable. Further investigations have shown that each pattern refers to a single gene, called colour gene. Many colour genes are located in the sex chromosomes[5–8].

3. *Colour genes and modification genes*

Detailed phenogenetic experiments have shown that a colour gene is responsible for at least 3 important orders: In the first place, it determines the further differentiation of certain pterin-containing cells in large polyploid cells containing melanin, so-called macromelanophores[9–11]. Secondly, it causes the macromelanophores to flock and stay together. Thirdly, it gives rough instructions in which part of the body and in what kind of tissue differentiation of macromelanophores will take place.

A colour gene, however, tells nothing about how frequently macromelanophores differentiate from pterin-containing cells and how often cell division has to occur in order to form and preserve the colour pattern typical for a given species. In addition it does not say at what time the macromelanophores differentiate, and it does not effect the polyploidy. Although it roughly determines the regions in the body where macromelanophores are formed, it does not limit them to that area. These orders and some in addition[12–14], important to the specific colour pattern as well as to the orders of the colour gene, are given by other genes. These genes, called 'modification genes' or 'modifiers', are located in gene systems, so-called 'modifier systems'[5]. The modi-

* Lecture given at the 'Internationaler Herbstkongress für Ganzheitsmedizin und Naturheilverfahren 1964' in Velden/Wörther See (Austria).

[1] J. HUXLEY, *Biological Aspects of Cancer* (Allen and Unwin, London 1958).

[2] W. J. BURDETTE, Cancer Res. *12*, 201 (1952); M. L. KAPLAN, Bull. Brooklyn ent. Soc. *51*, 1 (1956); R. C. KING, R. G. BURNETT, and N. A. STALEY, Growth *21*, 239 (1957); G. RÖHRBORN, Wilhelm Roux' Arch. Entw Mech. *150*, 115 (1957); J. H. SANG and B. BURNET, Genetics, Princeton *48*, 235 (1963); and others.

[3] A. E. KEHR, Am. Nat. *85*, 51 (1951); A. E. KEHR and H. H. SMITH, Brookhaven Symp. Biol. *6*, 55 (1954); U. NÄF, Growth *22*, 167 (1958); F. ANDERS and F. VESTER, Experientia *16*, 65 (1960); F. VESTER and F. ANDERS, Biochem. Z. *332*, 396 (1960); H. H. SMITH, Ann. N.Y. Acad. Sci., Ser. 2, *24*, 741 (1962); E. STRITZ, Diss. Univ. Saarbrücken 1963; Experientia *21*, 647 (1965); M. R. AHUJA, Q. Rev. Biol. *40*, 329 (1965).

[4] Simultaneous discoveries and first investigations by: M. GORDON, Genetics *12*, 253 (1927); C. KOSSWIG, Z. indukt. Abstamm.- u. Vererb. Lehre *44*, 253 (1927); G. HÄUSSLER, Klin. Wschr. *7*, 1561 (1928).

[5] Last reviews: M. GORDON, *Pigment Cell Biology* (Academic Press, New York 1959), p. 215; C. KOSSWIG, Copeia *1964*, 65 (1964).

[6] C. KOSSWIG, Experientia *20*, 190 (1964); Naturw. Rdsch. Stuttg. *18*, 392 (1965).

[7] J. W. ATZ, Zoologica N.Y. *47*, 153 (1962); K. D. KALLMANN, Zoologica N.Y. *50*, 151 (1965).

[8] F. ANDERS and K. KLINKE, Z. VererbLehre *96*, 49 (1965).

[9] H. BREIDER, Strahlentherapie *88*, 619 (1952).

[10] I. ZIEGLER, Z. Naturf. *18b*, 551 (1963) Ergebn. Physiol. *56*, 1 (1965); W. PFLEIDERER and E. C. TAYLOR, *Pterine Chemistry* (Pergamon Press, Oxford, London, Edinburgh, New York, Paris, Frankfurt 1964), p. 485.

[11] G. MAAS, Diss. Univ. Saarbrücken (1966).

[12] To this belongs also the decision of whether and how much melanin is produced. But pigment formation will not be considered, because it is dependent on a separate genetic make-up that does not effect tumour formation: albino fish are able to form complete melanomas lacking melanin.

[13] H. BREIDER, Z. wiss. Zool. *152*, 107 (1939).

[14] F. ANDERS, F. VESTER, K. KLINKE, and H. SCHUMACHER, Biol. Zbl. *81*, 45 (1962).

fiers are irregularly distributed in many or all autosomes. Modifier system and colour gene are in such a perfect agreement by natural selection that in almost every case a species-specific pattern is formed depending on the colour gene.

In the following a colour pattern is described.

4. *Colour pattern of a wild Mexican* Platypoecilus maculatus

Figure 1 shows at top left a female of a wild Mexican *Platypoecilus maculatus*. In the cutis of the dorsal fin are little black spots. Each spot consists of about 100–300 compactly packed macromelanophores[11] which are determined by the colour gene *Sd* ('spotted dorsal') located in the *X*-chromosome. However, the spots are not present at birth. The first colour cells appear not before the second or third month of life. By continuing differentiation and cell division, more are formed and joined to others, thus building the spot pattern. In general, during the whole of life colour cells are formed so that the size of the spots grows continually[14]. The spots of the fish shown have almost reached the final stage. They are nearly in agreement with those in all wild animals of the same age and genotype. For that reason the colour gene and the corresponding modifier systems seem to be one and the same genetic factor, although this is not true.

5. *Tumour formation caused by displacement of colour-specific modifiers*

As long as the *Sd*-spots appear in the purebred *P. maculatus* from Mexico, they never form melanomas. This occurs only in hybrids between certain subspecies of *P. maculatus*, and between *P. maculatus* and certain other species, for example *Xiphophorus helleri*, the wild 'swordtail' (Figure 1, at top right)[15].

The colour pattern of *X. helleri* is different from that of *P. maculatus*. Colour spots in the dorsal fin like those in *P. maculatus* (Figure 1, at top left) are never found in *X. helleri* (Figure 1, at top right). Therefore the species *X. helleri* does not possess the *Sd*-gene and the corresponding modifier systems. The colour pattern of *X. helleri* is controlled by its own colour genes and modifier systems which are in good agreement obtained by natural selection during evolution. For better understanding, this colour pattern will be neglected in the following.

In crossbreeds of *P. maculatus* (Figure 1, at top left) with *X. helleri* (Figure 1, at top right) the colour gene *Sd* loses half of its modifiers because of the splitting of genes or chromosomes obeying Mendel's laws. The genes replacing the *Sd*-modifiers have by reason

[15] Various species are now crossed by artificial insemination; C. D. ZANDER, Zool. Anz. *166*, 81 (1961).

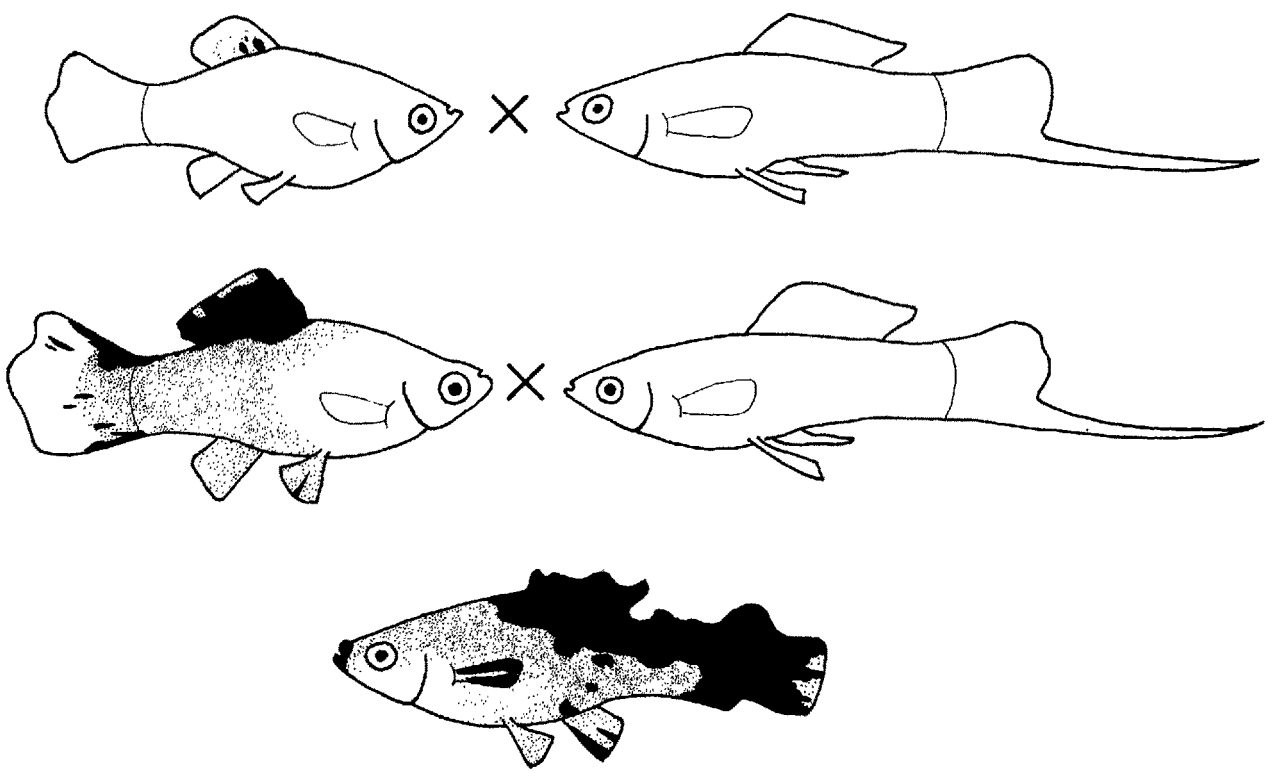

Fig. 1. First row: *Platypoecilus maculatus* (*Sd* = Spotted dorsal and *Dr* = Dorsal red) × *Xiphophorus helleri*. *Sd* and *Dr* in *P. maculatus* are repressed. Second row: *Sd*, *Dr* F_1-hybrid × *X. helleri*. *Sd* and *Dr* in the hybrid show partial derepression and increased induction. Third row: *Sd*, *Dr* back-cross hybrid. *Sd* and *Dr* show total derepression and increased induction. See text.

of their origin other functions, and they are, therefore, no compensation. This has radical consequences for the phenotype: the F_1-hybrids possess already at the end of the first month *Sd*-macromelanophores (about 1–2 months earlier than *P. maculatus*). During further development thousands of highly polyploid (up to 16 *n*) colour cells are formed by continuous differentiation and cell division[11], so that in the cutis of the dorsal fin the macromelanophores accumulate (Figure 1, second row left). Colour cells, for which there is obviously no space in the dorsal fin, migrate to other areas of the body which are commonly determined by the colour gene, by the colour gene-specific modifiers, and by the colour gene-unspecific genes originated from *X. helleri*. Thus these regions are colour gene and hybrid-specific. At first the dorsal regions of the caudal fin and then the regions between dorsal and caudal fin are occupied by colour cells; extremely, the colour cells are found in the ventral and finally in the middle regions of the caudal fin. The formation of the colour cells continues at the same rate, and if, in the assigned areas, the capacity of the cutis is exceeded, the colour cells will start growing in the third dimension: from spots are formed tumours. In our example, this change from spot to tumour is normally reached in the second half of life[14]. Continuously growing melanomas are formed, which show all histological, cytological and biochemical peculiarities of real tumours[11,16]. Yet the growing of the tumours in these F_1-hybrids is relatively slow and is reduced during senescence. In general, the normal duration of life of the fish is therefore not noticeably reduced.

The fertility of these hybrids is almost unrestricted, and permits further crossbreeds. In this case, firstly, the back-cross of F_1-hybrids with *X. helleri* (Figure 1, second row), is interesting because in this cross *Sd*-specific modification genes are replaced again by *Sd*-unspecific genes. In the following back-crosses of the back-crossed hybrids with *X. helleri*, the elimination of the modifiers is completed, so that all autosomal *Sd*-specific modification genes are replaced. Now the phenotypical effect of the elimination is of great consequence (Figure 1, third row): all back-cross hybrids carrying the *Sd*-gene form highly polyploid macromelanophores already before or shortly after their birth. Beyond this, the frequency of colour cell formation increases abnormally, so that immediately all areas already described with regard to F_1-hybrids are now occupied by newly-formed colour cells. A few weeks later the spots change to fast-growing melanomas. The tumours, now growing in several areas, melt together and form one large connected melanoma. While this is going on, still other colour gene-specific and hybrid-specific regions are occupied by dividing colour cells: mouth, pectoral fins, and some regions distributed over the sides of the body. Finally the colour cells are able to penetrate the body of the fish, except for the nervous system, intestines, and skeleton, and can amount to about 65% of the weight of the body. Now fish and tumour growth have come to an end, and the fish dies[5,7,14].

6. *Prevention of tumour formation by addition of colour gene-specific modifiers*

In contrast to this, the back-cross of the tumour-forming *P. maculatus*/*X. helleri*-hybrids with *P. maculatus* has other consequences. In this case the colour gene manifestation is successively normalized by stepwise introduction of the *Sd*-specific modifiers. The colour gene again causes normal spot pattern, even when the colour gene has determined tumour formation for many generations[17].

7. *Repression and derepression of the colour gene*

Without discussing any details of *Sd*-caused tumour genesis, the genetic make-up which is responsible for the formation of the colour pattern and tumours will be considered again.

The results of crossbreeds have shown that the colour gene *Sd* causes an unlimited and abnormally fast differentiation[18] and multiplication of macromelanophores, if it lacks its own modifier system. It seems to possess an elementary capacity of potentially unlimited activity. Only the modifier systems which are made in accordance with the colour gene by natural selection are able to reduce and concentrate its activity on the formation of a typical colour pattern. In this sense the modifiers discussed above are also 'repression genes'[8,19], and thus undoubtedly have the same general functions which are characteristic for the so-called 'regulator genes' of microorganisms[20]. The formation of the colour pattern depends on repression, while the tumour formation depends on derepression of the potentially unlimited activity of the colour gene. Repression is caused by modification genes and derepression by its absence.

8. *A repression gene which occupies the first place in preventing tumour formation*

Phenogenetical analysis of the repression genes has shown that these genes rank in order according to how

[16] H. Breider, Z. Zellforsch. mikrosk. Anat. *28*, 784 (1938); D. G. Humm and J. H. Humm, Ann. N.Y. Arad. Sci. *100*, 857 (1963).
[17] Till now a maximum of 15 generations.
[18] M. Gordon (see [5]) discussed the fact that differentiation of pigment cells in tumours is an incomplete one; but tumour cells are real pigment cells producing melanin.
[19] F. Anders, Verh. dt. zool. Ges., Kiel (1964), Zool. Anz. *28*, Suppl.-Bd., 102 (1965).
[20] F. Jacob and J. Monod, in *Cytodifferentiation and Macromolecular Synthesis* (Ed. M. Locke; Academic Press, New York and London 1963), p. 30; H. O. Halvorson, A. Herman, H. Okada, and J. Gorman, 13. Colloquium der Ges. für physiol. Chemie in Mosbach/Baden (Springer, Berlin-Göttingen-Heidelberg 1963), p. 42.

strongly they prevent tumour formation. The first place is occupied by a certain repression gene (RG_1), that obviously represses only the formation of colour cells. It is located in one of the autosomes, following Mendel's laws[21]. If 2 of them are present in the cell, tumour formation is always prevented. This dosage allows the colour gene only a relatively small activity (Figure 2a). A single dosage of the repression gene allows a relatively great activity of the colour gene, mostly resulting in tumour formation (Figure 2b), while its absence allows a very high activity of the colour gene, that is very high tumour formation (Figure 2c). Modifiers determining the limits of the areas or modifiers determining polyploidy etc., are obviously unimportant for the prevention of tumour formation. Their absence has only a modifying influence on tumour formation.

9. *Influence of colour gene-unspecific modification genes on the activity of the colour gene*

After discussing colour gene-specific modifiers, we should like to introduce colour gene-unspecific modification genes.

These modifiers can be recognized by crossing *P. maculatus* with various geographical races of *X. helleri* or other species of the genus *Platypoecilus* or *Xiphophorus*[8,19,22]. Using a race of *X. helleri* living in the Rio Papaloapan (Mexico), relatively intensive tumour formation can be obtained[23]. If this race is substituted by *X. helleri* living in the Belize River (British Honduras), we find very little tumour formation, often showing only an increased spot pattern. If *X. helleri* is replaced by *X. montezumae cortezi*, this results in a more intensive tumour formation than in the crossbreeds of *P. maculatus* with *X. helleri* living in the Rio Papaloapan. Crossbreedings with other species again have different results, for example an increased or normal formation of colour spots.

Interesting too, is the following example giving more information: After crosses and back-crosses of *P. maculatus* with *P. xiphidium*, the colour gene *Sd* completely stops its activity, although the *Sd*-specific repression genes have been displaced. In this case no colour cells will be determined at all. Already these examples show that *X. helleri* and the other species have genes influencing the activity of the *Sd*-colour gene of *P. maculatus*. In this sense they are non-species-specific modifiers of the *Sd*-gene. However, since they originate from species which have developed no *Sd*-gene during their evolution, these modifiers cannot be *Sd*-specific. It is more likely that they belong to the genetic milieu of a certain species, and that they have general functions. Of course, *P. maculatus*, too, has such modification genes; but the *Sd*-gene is made so well in accordance with its own genetic milieu by natural selection that the spot formation is always constant. For this reason the influence of the genetic milieu on its own colour gene cannot be recognized. The genetic milieu of *P. maculatus* can only be recognized if it is combined with non-species-specific colour genes. Under this condition the genetic milieu of *P. maculatus* influences the non-species-specific colour genes in the same manner as the *Sd*-gene of *P. maculatus* is influenced by the genetic milieu of *X. helleri*.

Till now this type of modification gene could be analysed as a species-specific unit of gene systems.

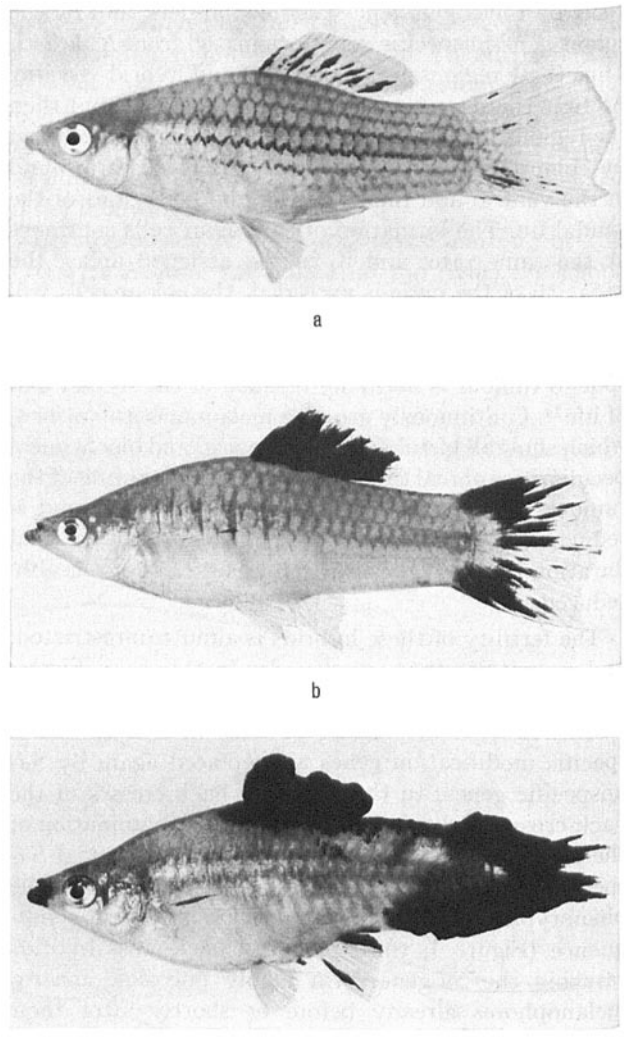

Fig. 2. (a) *Sd, Dr P. maculatus/X. helleri* hybrid possessing twofold dosage of the repression gene RG_1. (b) *Sd, Dr P. maculatus/X. helleri* hybrid possessing single dosage of the repression gene RG_1. (c) *Sd, Dr P. maculatus/X. helleri* hybrid without repression gene RG_1. See text.

[21] F. ANDERS and K. KLINKE, Verh. dt. zool. Ges., Göttingen, (1966) in print.

[22] C. D. ZANDER, Mitt. hamb. zool. Mus. Inst. *60*, 205 (1962); Mitt. hamb. zool. Mus. Inst., *Kosswig-Festschrift* 222 (1964).

[23] This race was used in Figure 1.

Analysis of simple modifiers is very difficult because of the complexity of the genetic milieu[24]. Since the discovery of these genes by KOSSWIG in Germany (1927) and GORDON in the United States (1927)[4], no one doubted their existence[7,8,16,22,25,26].

The following observations have a key position in understanding the efficiency of this type of modification gene.

10. *Influence on the activity of the colour gene by the environment*

While the manifestation of the colour gene in the purebred species is nearly stable to environmental changes, it becomes more and more unstable after hybridization, by which the repression genes are successively displaced[8,14]. In general, the colour gene reacts upon environmental stimulators only by forming colour cells at a higher rate. These environmental stimulators are: more than optimal feeding, small number of individuals in a population, lowered water temperature, increased saline concentration of the water, low UV-irradiation, small doses of chloramphenicol, steroid hormones, and other factors, which are all obviously stimulating the growth of the body. They give the impression of a better physical condition of the fish, although the number of broods and the number of offspring per brood are reduced, and often propagation ceases.

Influenced by the factors mentioned above the rate of colour cell formation can be greatly increased. In a certain combination of genes the number of colour cells could be increased 35-fold during 7 days[27]. Working with tumour-forming hybrids the tumour appears earlier than normally, and the rate of tumour formation is increased. These fishes die several months earlier than the animals in the control experiment. Colour spot forming hybrids show tumour formation, and hybrids forming no colour cells or spot pattern now show formation of colour spots.

Strangely enough, relatively short exposure of the animals to the environmental stimulators mentioned above is sufficient to raise the expression of the colour gene (e.g. sub-lethal increase of saline concentration during 30 days); for this it does not matter whether the factors are acting during the early oogenesis (e.g. temporary exposure of the mother to diluted sea water), or embryogenesis, juvenescence or senescence[27]. The activity of the colour gene, increased by changing the environment for a short period, lasts more than a year, and therefore gives the impression that this increased activity is irreversible[28]; only very much later does the increased colour cell formation gradually fade. In addition, it is not possible to reduce colour cell formation (or tumour formation) to a normal rate by changing the environment to the opposite (e.g. insufficient feeding, raising water temperature etc.). The environmental factors are able to reduce colour gene manifestation only when they act continuously from early oogenesis. The colour gene does not even show any reaction to short environmental stimulations which are believed to reduce colour cell formation.

11. *Colour gene-unspecific modification genes and environmental factors as inductors of colour gene activity*

The result of section 10 is: the activity of the colour gene depending on non-species-specific modifiers can be raised by environmental stimulators. This state is still retained after the stimulation is finished. This also shows that the non-species-specific modifiers are not repressors, even in those gene combinations in which the colour gene normally does not induce colour cell formation at all; its activity is not prevented by repression, because it can be stimulated by temporary environmental changes to form colour cells continuously[29]. In this case the stimulus of the modifiers is obviously lacking, or not strong enough to make the colour gene form colour cells. In derepressed genotypes in which colour gene manifestation is present, the non-species-specific modifiers, however, induce a certain activity of the colour gene.

The potential elementary activity of the colour gene obviously requires an induction in order to be expressed. This induction is caused by colour geneunspecific modification genes which will be called 'induction genes', and by environmental factors.

12. *Amino acids, supposed to be an intermediate in induction of colour gene activity by modification genes*

Since colour cell formation in fish lacking repression genes is modified in the same manner either by induction genes of a non-species-specific genetic milieu or by heterogeneous environmental factors, let us assume that in the mechanism of all these factors a central link is involved. Among the substances which could represent such an intermediate, amino acids take the first place. These compounds can be extraordinarily

[24] C. KOSSWIG, Veröff. Inst. Meeresforsch. Bremerh., Sonderband *3*, Meeresbiol. Symp. 178 (1963).
[25] M. ÖKTAY, Mitt. hamb. zool. Mus. Inst., *Kosswig-Festschrift* 133 (1964).
[26] P. A. MACINTYRE and K. F. BALSER-COHEN, Zoologica *46*, 125 (1961).
[27] F. ANDERS, A. ANDERS, and K. KLINKE, Verh. dt. zool. Ges., Wien (1962), Zool. Anz. *26*, Suppl.-Bd., 97 (1963).
[28] F. ANDERS, F. DRAWERT, K. KLINKE, and K. H. REUTHER, Experientia *19*, 219 (1963).
[29] Some exceptions are known which will be examined here.

active in physiological or genetical mechanisms[30–32], either as exogenous or endogenous 'free' amino acids. A good example of this is the induction of galls by phytopathogenic insects using free amino acids[31], which have a regulative effect on the chain of physiological reactions leading from DNA to RNA, to the morphologic character[28]. They obviously have a gene regulative function in microorganisms, too[33].

Supposing that the free amino acids are a link in the functional mechanism of the induction genes, then the races, species, and hybrids mentioned in section 9—their *Sd*-unspecific induction genes cause different degrees of activity of the derepressed *Sd*-gene—should have different levels of amino acids. These differences in amino acid content should also be reflected in colour gene manifestation. The assumption could be proved experimentally, because the relative manifestation of the derepressed gene is correlated with the amino acid content of its carrier and of the races and species used in the crossbreeding. To what extent the so-called 'pattern' of the free amino acids plays a role, is uncertain at this time[14].

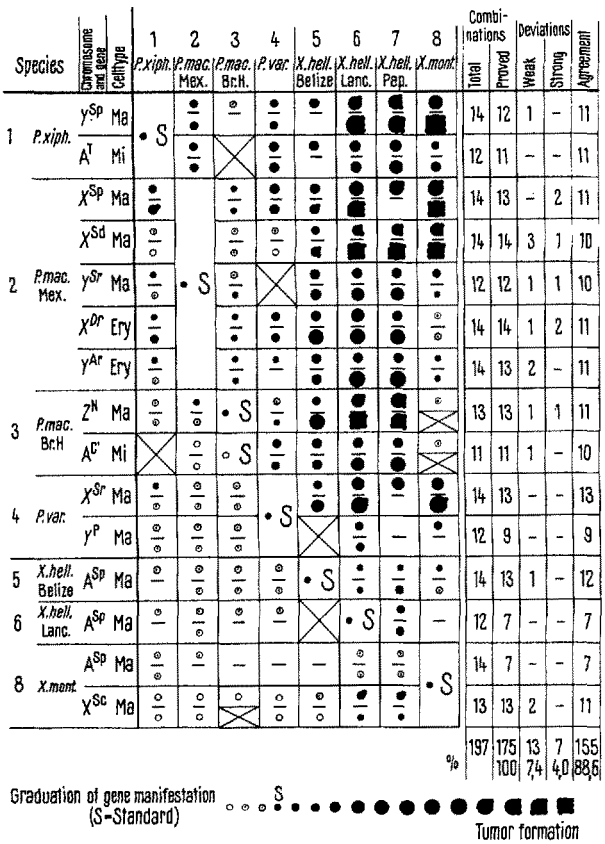

Fig. 3. Generalized manifestation of 15 colour genes out of 7 species or subspecies in 175 hybrid combinations between 8 species or subspecies (number 7, *X. helleri* from Rio Papaloapan, has no colour gene causing spots). Row 1–8 is based on the amino acid content. Various degrees of gene manifestation can change continuously from low to middle, to increased and much increased tumour formation. This is shown by the size of the black symbols and the number of protuberances (1–4) of the symbols. The number of protuberances shows not only the degree but also the frequency of tumour formation: 1 = less than 100%, 2–4 = 100% frequency of tumour formation. The upper of the 2 symbols in one square represents gene manifestation of the F_1-hybrids, and the lower one that of the back-cross hybrids; lacking symbol = not yet proved; ⊠ = proving is not possible. Sp = Spotted; T = Twin spot; *Sd* = Spotted dorsal; *Sr* = Stripe-sided; *Dr* = Dorsal red; Ar = Anal red (probably the same gene as Rb = Red belly as described by GORDON). N = Nigra (large black spots); C' = Crescent (in the caudal fin; C' is not the same as C = Crescent as described by GORDON); P = Punctatus (little spots at the side of the body); Sc = Spotted caudal. Ma = macromelanophores; Mi = micromelanophores; Ery = erythrophores = pterinophores. A = autosome; *X*, *Y*, *Z* = heterochromosomes; x = Autosome, which is homologous to *X*. See text, details in reference [8].

13. *Amino acids, supposed to be an intermediate in induction of colour gene activity by environmental factors*

While the amino acid content of fishes of the same genotype, age and sex are constant under constant

[30] See E. ROBERTS and D. G. SIMONSEN, in *Amino Acids, Proteins and Cancer Biochemistry* (Ed. I. T. EDSALL; Academic Press, New York and London 1960), p. 121; T. J. HOLDEN, *Amino Acid Pools* (Elsevier, Amsterdam-London-New York 1962), p. 248.

[31] F. ANDERS, Verh. dt. zool. Ges., Erlangen (1955), Zool. Anz. *19*, Suppl.-Bd., 421 (1956); Vitis *1*, 121 (1957); Biol. Zbl. *80*, 199 (1961); O. HENKE, Phytopath. Z. *41*, 387 (1961); G. SCHÄLLER, Zool. Jb., Allg. Zool. Physiol. *70*, 399 (1963); M. S. MANI, *Ecology of Plant Galls* (Dr. W. Junk Publishers, The Hague 1964).

[32] W. KLOFT, Z. angew. Ent. *46*, 42 (1960); G. L. HAGEN, Devl. Biol. *4*, 569 (1962); D. F. S. TRUMAN and A. KORNER, Biochem. J. *83*, 588 (1962); P. H. REIS and P. G. SCHINCKEL, Aust. J. biol. Sci. *16*, 218 (1963); F. C. STEWARD, Scient. Am. *209*, 104 (1963); G. WERZ, Planta *60*, 211 (1963); F. ANDERS, F. DRAWERT, A. ANDERS, and K. H. REUTHER, Z. Naturf. *19b*, 495 (1964); J. ŘEŘÁBEK, Naturwissenschaften *51*, 67 (1964); F. DRAWERT, K. H. REUTHER, and A. and F. ANDERS, Experientia *21*, 618 (1965).

[33] F. GROS and F. GROS, Biochem. biophys. Acta *22*, 200 (1956); M. YCAS and G. BRAWERMAN, Archs Biochem. *68*, 118 (1957); A. I. ARONSON and S. SPIEGELMAN, Biochem. biophys. Acta *53*, 70 (1961); F. C. NEIDHARDT and D. G. FRAENKEL, Cold Spring Harb. Symp. quant. Biol. *26*, 63 (1961); E. KELLENBERGER, K. G. LARK, and A. BOLLE, Proc. natn Acad. Sci. U.S.A. *48*, 1860 (1962); O. MAALØE and N. O. KJELDGAARD, *Control of Macromolecular Synthesis* (W. A. Benjamin Inc., New York and Amsterdam 1966).

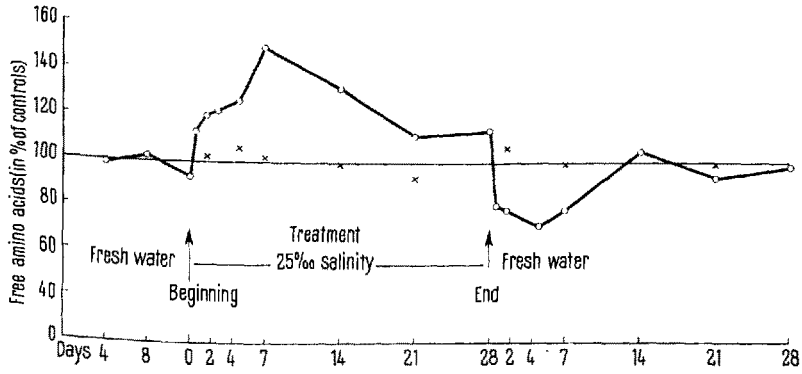

Fig. 4. Influence of diluted sea water on the amino acid level of *P. maculatus* from Mexico. 192 measurements with 50 animals; mM % = 2.3; from ANDERS et al.[14,27].

conditions, they are increased by the same environmental factors which stimulate the derepressed colour gene to higher activity[34]. Figure 4 shows the influence of diluted sea water on the amino acid content of *P. maculatus* of the same age and sex[35]. If the animals have a perfect set of repression genes, colour gene manifestation is almost not influenced by environmental stimulation (Figure 5, A). Hybrids, however, carrying a derepressed colour gene react upon raising the amino acid level by an increased colour cell formation for a long period (Figure 5, B and C; compare with section 10).

Thus amino acids possess the qualities demanded for a central link involved in the functional mechanism of induction genes and environmental factors[36].

14. *Dosage of colour gene*

In all the preceding examples the colour genes are present in single dosage. In what manner does the twofold dosage manifest itself?

In the beginning it should be said that most diploid organisms have developed a genetically fixed 'dosage compensation' for those genes which can be present in the organism in single or twofold dosage. By this compensation mechanism the dosage of the gene is stabilized so that the phenotypes possessing either the single or the twofold dosage of the gene are identical[37]. This is also true for the colour genes of the *Poeciliidae*. This, however, can be recognized only when the colour gene is regulated by its own repression genes. By stepwise elimination of the latter, the so-called 'dosage effect' can be seen, that means the twofold dosage of the colour gene has a twofold effect[21]. Tumour formation and death, already induced by the single dosage, are speeded up by the twofold dosage. Death often occurs during the first days of life, and in many breeds the number of individuals per brood possessing the twofold colour gene dosage is reduced. More intensive spot formation caused by single dosage results in tumour formation, if the twofold dosage is present.

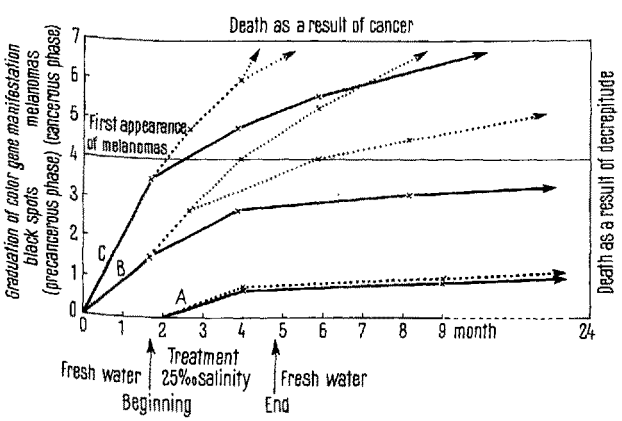

Fig. 5. Colour gene (*Sd*) manifestation in fresh water (drawn-out line) and under treatment with diluted sea water for 3 months (dotted line). A = repressed colour gene; B = partial derepressed colour gene; C = total derepressed colour gene. See text; combined from ANDERS et al.[14].

15. *Joint regulation of genes determining the formation of melanophores and erythrophores*

Melanophores originate from pterin-containing cells as already mentioned in section 3. The results of several investigators seem to prove that the formation of melanophores is connected with pterin metabolism[10]. From this point of view the following observations are interesting.

Till now we know at least 6 genes for formation of macromelanophores which are closely linked to genes forming pterin-containing colour cells (erythrophores).

[34] Till now only the effects of various salt concentrations, temperatures, and UV-irradiation have been investigated.

[35] The curve resembles that of technical regulating systems and therefore points to the presence of a biological regulating system that causes a constant concentration of the amino acids at different salt concentrations. See K. STEINBUCH, *Automat und Mensch* (Springer, Berlin-Heidelberg-New York 1965).

[36] Experiments for raising pigment cell formation by injecting amino acids are difficult to perform. Single injections are compensated, as already known for other objects. Continuous injections which would raise the level of amino acids for a longer period of time are not tolerated by the animals.

[37] C. STERN, Biol. Zbl. *49*, 261 (1929); H. J. MÜLLER, Proc. 6th Int. Conf. Genet. *1*, 213 (1932). Last review: A. G. COCK, Genet. Res. *5*, 354 (1964).

To these genes determining the formation of macromelanophores also belongs the colour gene *Sd*; the directly following gene in the chromosome is the *Dr*-gene ('Dorsal red', reddish glimmering of the dorsal fin; Figure 1, at top left, dotted area), which determines the formation of erythrophores. It is difficult to imagine that the genes responsible for the formation of macromelanophores happen to be jointly linked to genes responsible for the formation of erythrophores. It is more likely that this connection is the result of physiological relation between pterin metabolism and the formation of melanophores. The connection between a macromelanophore gene and an erythrophore gene is finally emphasized by their parallel manifestation in most cases (compare black and dotted areas in Figure 1). Obviously, their activity is mostly regulated by the same factors. This suggests the assumption that they represent something like an 'operon' in the sense of JACOB and MONOD[20].

16. *Attempt to represent a scheme of the genetical-physiological make-up that determines spot and tumour formation*

After several authors have tried to explain gene regulation in higher organisms in the sense of the well-known scheme of galactose utilization in *Escherichia coli*[38], an attempt will be made to explain the regulation of colour genes in platyfishes and swordtails in the same sense.

For simplicity's sake, we shall consider the example already described: *Sd* and *Dr* of *P. maculatus* in the purebred species and after hybridization with *X. helleri*. Since this example in general is valid for other colour genes and other poeciliid species, except for a few details (linkage, intensity of colour gene manifestation etc.), the symbolization will be generalized, too. In Figure 6a all factors are summarized which guarantee the normal manifestation of *Dr* (CG_1) and *Sd* (CG_2) in the purebred species *P. maculatus* (see Figure 1, at top left). Colour genes and their linkage to the *X*-chromosome have been the object of many investigations and are therefore well known. In most cases they are jointly regulated. Also well known is the autosomal repression gene (RG_1) which has a key position in the repression of colour cell formation. It turned out to be specific for CG_1 and CG_2. Other repression genes (*RG*) have been found by analysing modifier systems. Probably, they are irregularly distributed among some or all autosomes ($2n = 48$). Whether there are gonosomal repression genes too is not certain[21]. From our knowledge of gene function, we have to postulate specific repressor substances (*RS*), especially in these fishes, because the repression genes and the colour genes are located in different chromosomes. Nothing is known definitely about the repressor substance. Till now induction genes (*IG*) are only known as additive components of polygenic systems of the genetic milieu. They are obviously distributed among many or all chromosomes. There is circumstantial evidence of the identity of the induction genes and those genes controlling the amino acid pool (AA-pool), and also of the inductional effect of the amino acids (see scheme). The induction influences the colour genes and causes the genes to differentiate erythrophores (Ery) and macromelanophores (Ma). As common metabolites, the amino acids are of course involved in the entire formation of colour cells (see right arrow in the scheme). The concentration and the pattern of the free amino acids are, as we know for certain, determined by genes and can be influenced by environmental factors. The interaction of all the factors already mentioned (and probably several more factors) causes the differentiation of a definite number of red and black colour cells arranged to the species-specific colour pattern: black spots on a reddish glimmering background (Figure 1, at top left). Because the spots increase in size slowly till death of the individuals, it is not necessary to postulate feedback-reactions from spots to the factors which differentiate them.

A corresponding scheme for *X. helleri* from Rio Papaloapan (Figure 1, at top right) is shown in Figure 6b. Colour genes determining the formation of macromelanophores and macromelanophore gene-specific repression genes are lacking. Genes recognized as induction genes (*IG*) in the cross *P. maculatus*/*X. helleri* cause a higher level of free amino acids than the corresponding genes of *P. maculatus*. The characteristics of the F_1-generation (Figure 1, second row left) can be seen in Figure 6c. These are: low repression or partial derepression and increased induction causing increased formation of erythrophores and melanophores. The colour spots of the pattern change to melanomas (see irregular black spot in the scheme).

In Figure 6d are shown all the characteristics of the back-cross (Figure 1, third row). More increased formation of erythrophores and melanophores is caused by total derepression or lacking repression and by high induction. The fishes have black melanomas on red background (see large irregular black spot in the scheme).

These expressions of the colour genes are increased again if the fishes possess the twofold dosage of the genes (see Figure 6e). If both of the parents possess one or several colour genes, the offspring show a more complicated genotype and more differentiated characteristics. But there are no new aspects.

17. *Concluding remarks*

The formation of melanomas in platyfish-swordtail-hybrids is caused by partial or total derepression and

[38] B. MCCLINTOCK, Am. Nat. *95*, 265 (1961); H. MARQUARDT, Biol. Zbl. *83*, 1 (1964); and others.

increased induction of a gene which determines a certain type of cell. Since cancer generally is probably based on disordered cell growth[39], the causes are perhaps universal.

By contrast, the constitution of causes might not be universal. The melanomas in *Poeciliidae* originate from recombination in the spermovium resulting in derepression and induction. The same is true for specific tumours of the thyroid gland and erythrophoromata (tumours consisting of erythrophores) of these fishes as well as for tumours in tobacco-hybrids[40]. Readiness for tumour formation often found in warm-blooded animals might be constituted similarly. By contrast, in most of the tumours of warm-blooded animals—today the most favoured object of investigation—the constitutions of causes are obviously not recombination in the zygote but other mostly unknown origins.

Our knowledge today about the origin of the causes of fish melanomata allows us to discuss some possible constitution of causes of tumours in warm-blooded animals:

Repression genes which can be eliminated in *Poeciliidae* by crossing could have been deleted or mutated in warm-blooded animals in each germ cell and each somatic cell. Induction genes could have mutated at any time too, to a state in which they attain epistasis over the repression genes. Genes determining a certain type of cell could have undergone mutation so that they could not be controlled by their repression

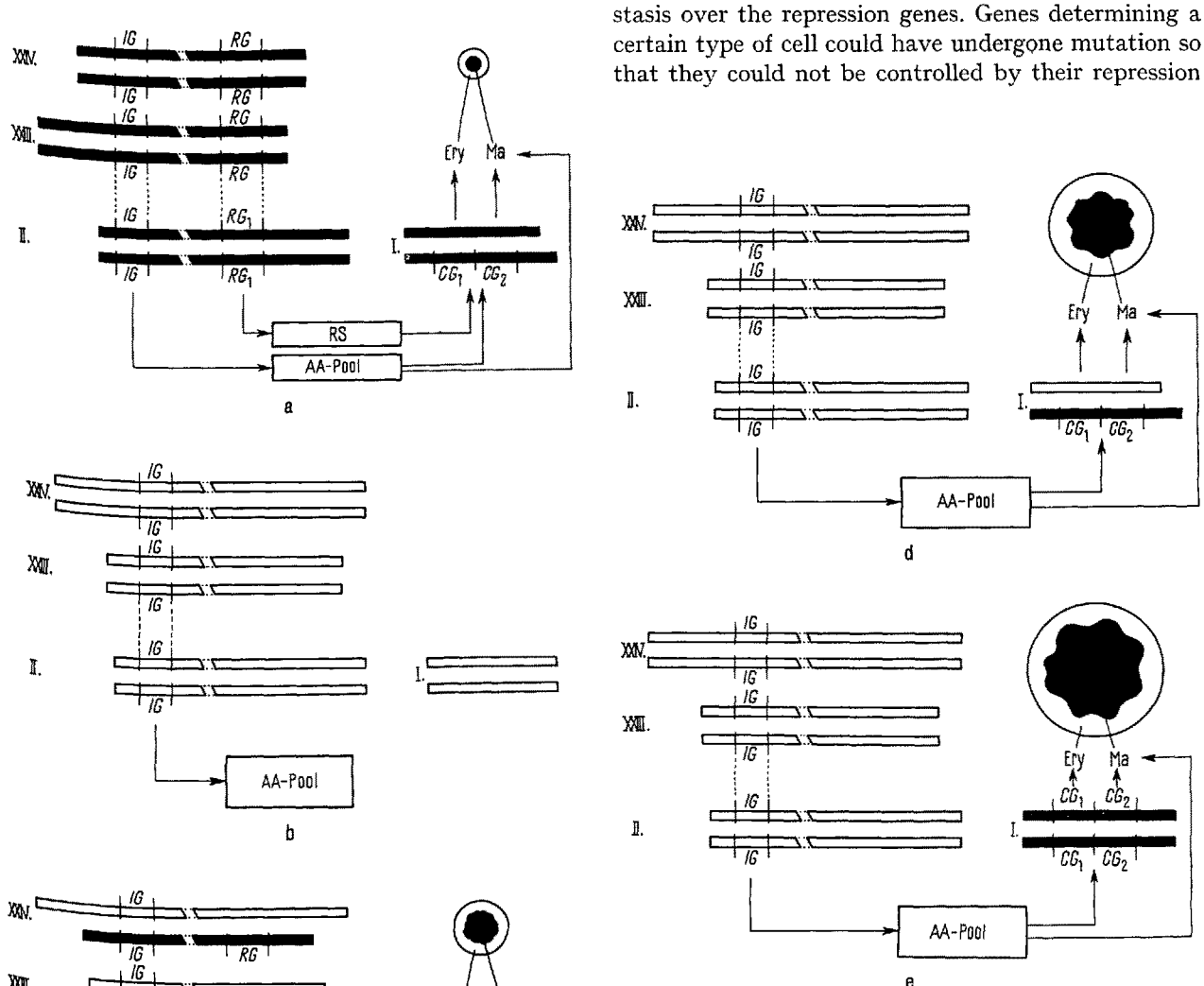

Fig. 6. See text of section 16. Chromosomes of *P. maculatus* = black; chromosomes of *X. helleri* = white.

[39] K. H. Bauer, *Das Krebsproblem* (Springer, Berlin-Göttingen-Heidelberg 1963), 2. Aufl.; A. Graffi and H. Bielka, *Probleme der experimentellen Krebsforschung* (Geest und Portig, Leipzig 1959).

[40] E. Steitz and F. Anders, Verh. dt. zool. Ges., Göttingen (1966), in print.

genes any more. Viruses and carcinogens could cause mutation or could block or disturb repression in another way. It might be possible that different blocks in different spaces are added, so that the sum of many defects, which in themselves are unimportant for tumour formation, could cause disordered cell growth.

From this point of view the formation of melanomas in *Poeciliidae* represents a model for the formation of cancer[41].

Zusammenfassung. Bei bestimmten Zahnkarpfen-Bastarden treten stets erbbedingte Melanome auf. Diese entstehen dadurch, dass bestimmte Gene, die für die Differenzierung von Farbzellen verantwortlich sind, enthemmt und gleichzeitig zu einer gesteigerten Aktivität angeregt werden. Die Enthemmung beruht auf einem Verlust bestimmter Repressionsgene und die Aktivitätssteigerung auf einer Einführung polyfaktorieller Systeme von Induktionsgenen. Es bestehen schwerwiegende Indizien dafür, dass die Induktorsubstanzen mit Aminosäuren identisch sind.

[41] I should like to thank Dr. C. KOSSWIG (Hamburg) and Dr. M. DZWILLO (Hamburg) for their valuable criticism, J. VIELKIND (Giessen) for translation, and W. BACKER (Giessen) for reading through the manuscript. The experiments of the author have been supported by a grant from Deutsche Forschungsgemeinschaft and Stiftung Volkswagenwerk.

SPECIALIA

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IR-Spectra of Fluorapatite and Fluorchlorapatite

Apatites are crystalline solids typified by hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, a basic calcium phosphate. Many substituents are possible in the apatite lattice giving rise to a whole series of related compounds.

It is now possible[1] to prepare an apatite with fluoride in the place of the hydroxyl ion, in which case the compound is known as fluorapatite, $Ca_{10}(PO_4)_6F_2$. The mixed apatites have also been prepared by heating together calculated quantities of β-$Ca_3(PO_4)_2$, $CaCl_2$ and CaF_2 at 800 °C or by treating a pure synthetic chlorapatite with different amounts of CaF_2.

When fluoride is fully substituted for all the hydroxyl ions in hydroxyapatite, the unit cell of the resulting fluorapatite has a smaller a-axis but the same c-axis as the unsubstituted hydroxyapatite. It is possible that this lattice parameter difference is due to the change centred in the calcium triangle. The OH ions of hydroxyapatite lie with their internuclear axis coincident with the sixfold screw axis and at a distance of 0.3 Å from the nearest trigonal calcium plane. On the other hand, the F ions of fluorapatite lie at the intersection of the plane with the sixfold screw axis, and the Cl ions of chlorapatite lie on the sixfold screw axis but midway between adjacent planes[2]. These positions are illustrated in Figure 1.

The first apatite structure completely worked out was that of the mineral calcium fluorapatite[3,4]. It is of hexagonal structure, having space group $P6_3/m$. The unit cell dimensions, for hydroxyapatite[5] and fluorapatite[4] are given in Table I.

The IR-spectra of hydroxyapatite, fluorapatite, and fluorchlorapatites are shown in Figures 2–6. The IR-spectrum of hydroxyapatite is included for reference purposes. The hydroxyapatite and other specimens were

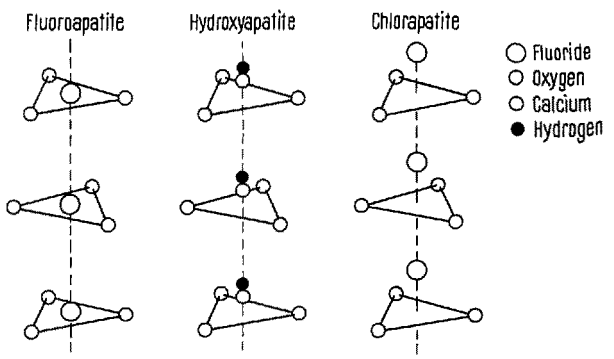

Fig. 1. Diagram showing the fluoride, hydroxyl, and chloride positions in the apatite structure[2].

Table I. Unit cell dimensions of hydroxyapatite[5] and fluorapatite[4]

Apatite	a Å	c Å
$Ca_{10}(PO_4)_6(OH)_2$	9.432 ± 0.005 Å	6.881 ± 0.005 Å
$Ca_{10}(PO_4)_6F_2$	9.37 ± 0.01 Å	6.88 ± 0.01 Å

[1] R. WALLAEYS and G. CHAUDRON, C. r. Acad. Sci., Paris *231*, 355 (1950).
[2] J. M. STUTMAN, J. D. TERMINE, and A. S. POSNER, Trans. N.Y. Acad. Sci. *27*, 669 (1965).
[3] M. MEHMEL, Z. Kristallogr. Miner. *75*, 323 (1930).
[4] S. NARAY-SZABO, Z. Kristallogr. Miner. *75*, 387 (1930).
[5] M. I. KAY, R. A. YOUNG, and A. S. POSNER, Nature *204*, 1050 (1964).