(pH 8.1) at 89–91 °C for 15 to 45 min. The slides were rinsed in water and stained with 2% Giemsa in distilled water for 15 min.

Metaphase chromosomes of cells which had completed 2 replication cycles in BrdU showed excellent differential staining between sister chromatids (Figure 1). The G_2-PCC cells which had finished the second DNA synthesis period showed SCD patterns similar to those of the metaphase chromosomes (Figure 2). The S-PCC (Figure 3) showed differential staining between sister chromatids of the replicated fragments and 'pulverized' regions indicating DNA synthetic activity immediately before the induction of PCC. The G_1-PCC (Figure 4) showed 2 types of staining patterns. Some chromosomes had differential staining along their lengths while others did not. The G_1-PCC pattern can be interpreted in the following ways. The cells of the cultures used were not synchronized and there is considerable variation in generation time, therefore the intra-chromosome differential staining seen in the cell in Figure 4 could be the result of sister chromatid exchanges in the 2 previous generations. Alternatively, the cell might have been in the S phase of the first cycle at the time of BrdU addition and progressed to the G_1 phase of the 3rd cell cycle at the time of PCC induction. This would result in parts of the G_1 chromosome(s) containing doubly substituted BrdU regions. The inter-chromosome differential suggests random sister chromatid segregation in the preceding anaphase.

Although the mechanism of SCD is still unknown, there is some evidence that unifilarly and bifilarly BrdU substituted chromatids have different packing densities observable by both phase and electron microscopy[6]. Since we have demonstrated that SCD can be induced in PCC much the same way as for mitotic chromosomes, it is probable that a similar density difference, as observed in electron micrographs, may exist among such differentially BrdU incorporated chromatids in PCC. This suggests an organizational similarity between PCC and metaphase chromosomes.

The combination of these 2 techniques will now allow greater precision in DNA synthesis[17] and chromosome aberration studies[18,19].

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Localization of Bovine Pancreatic Polypeptide (BPP)-Like Immunoreactivity in Rat Pancreatic Monolayer Culture

C. Rufener, D. Baetens and L. Orci[1]

Institut d'Histologie et d'Embryologie, Ecole de Médecine, CH-1211 Genève 4 (Switzerland), 9 February 1976.

Summary. Antiserum to bovine pancreatic polypeptide (BPP) has been used for immunofluorescent staining in the light microscope. With this technique it is possible to detect the presence of specific cells in monolayer culture from neonatal rat pancreas which contain BPP or a closely related peptide.

During the isolation of chicken insulin, Kimmel et al.[2] found a polypeptide containing 36 amino acid residues in a straight chain[3] and grouped in a sequence distinct from that of insulin, glucagon, somatostatin, secretin and gastrin. This avian pancreatic polypeptide (APP) has pronounced biological effects[4] and is a normal constituant of circulating plasma in chicken[5]. It was therefore postulated that APP represents a new pancreatic hormone[4,5] present in the cytoplasm of numerous cells disseminated in the exocrine chicken pancreas[6].

Recently, a mammalian counterpart of APP was isolated from the bovine pancreas and named accordingly bovine polypeptide (BPP)[7]. BPP has a sequence of 16 amino acid residues in common with APP and some similarities in biological action[7,8]. In the course of a systematic immunohistochemical study of the endocrine cell population of rat pancreatic monolayer culture, we were able to identify cells reacting to anti-BPP.

Neonatal rat pancreatic monolayer cultures were prepared as described elsewhere[9]. After 3 days of culture, tissue was fixed with a solution of 0,2% picric acid and 2% paraformaldehyde[10] in 320 mOs*M* phosphate buffer (pH 7,4)[11] during 2 to 90 h. After fixation, the cultures were washed in phosphate buffered saline, quickly dehydrated through increasing concentrations of ethanol, rehydrated and processed for indirect immunofluorescence[12]. Cultures were exposed to rabbit anti-BPP

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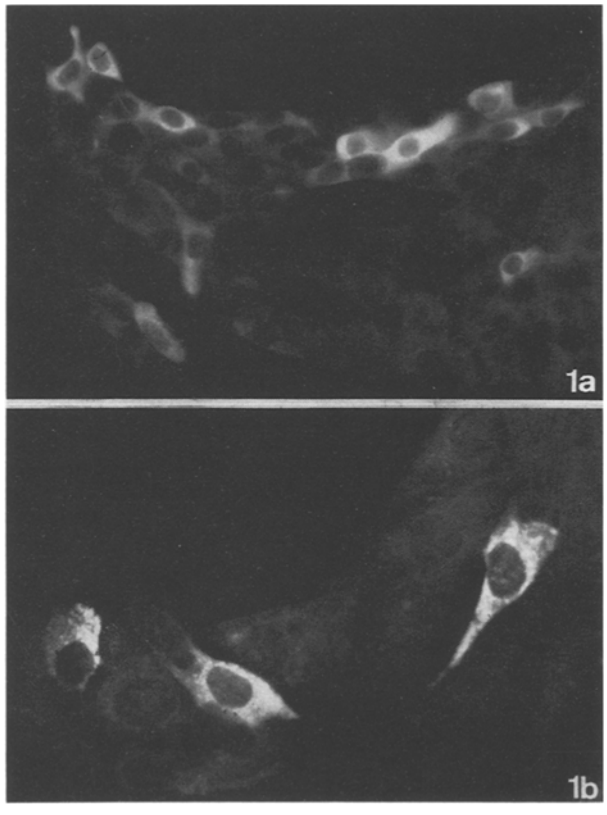

a) Three-day-old rat pancreatic monolayer culture incubated with anti-BPP serum, showing several fluorescent cells. ×400. b) Same culture as in (a), at higher magnification, showing 3 positive cells. Notice that the immunofluorescent reaction is confined to the cytoplasm. ×680.

(courtesy of R. E. CHANCE, Eli Lilly, Indianapolis, USA) diluted 1:10 in phosphate-buffered saline, then to a fluorescein labelled anti-rabbit γ-globulin serum (Pasteur Institute, Paris). Controls included adsorption of the specific anti-BPP serum with BPP (50 µg/ml of undiluted serum), insulin (2 U/ml), glucagon (50 µg/ml), somatostatin (200 µg/ml), secretin (50 µg/ml), gastrin (50 µg/ml) and GLI (90 µg/ml). All Petri dishes were counterstained with 0,01% Evans' blue after incubation and observed in a Zeiss Ultraphot microscope equipped with a III RS condensor.

After exposure to anti-BPP serum, strong immunofluorescence was observed in several cells (Figure, a–b) scattered throughout the endocrine clusters. No immunofluorescent cells were observed when anti-BPP was adsorbed with BPP prior to incubation. In contrast, the staining with anti-BPP serum was not abolished by adsorption of the serum with either insulin, glucagon, somatostatin, secretin, gastrin or GLI thus excluding any cross reactivity with these polypeptides.

From these results, it appears that a specific cell population of rat pancreatic monolayer culture contain a polypeptide identical or similar to BPP. Investigations are now in progress so as to identify at the ultrastructural level the cell type reacting with anti-BPP. BPP immunoreactivity was recently found in dog pancreas and gastrointestinal tract. Fluorescent cells were particularly numerous in the uncinate process scattered through the exocrine tissue, and may correspond to the so-called F-cell[13].

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Pluralité dans le déterminisme des effets tératogènes des composés organophosphorés

Plurality in the Determinism of Organophosphorus Teratogenic Effects

R. MEINIEL

Laboratoire de Biologie Animale, E.R.A.,C. N.R.S. 408, Université de Clermont, Boîte postale 45, F–63170 Aubière (France), 26 janvier 1976.

Summary. In Quail embryos, nicotinamide prevents beak and legs abnormalities produced by bidrin but remains inefficient against vertebral defects induced by bidrin and parathion. In contrast, the vertebral deficiencies are greatly alleviated or abolished by pralidoxim, an antidote known and used in organophosphorus intoxications. From these observations, a plurality in the determinism of teratogenic effects induced by organophosphorus compounds is evident.

Les insecticides organophosphorés, bien connus pour leurs propriétés anticholinestérasiques[1] sont de puissants tératogènes chez l'oiseau. Ils induisent des déformations de l'axe vertébral[2–4], des anomalies des membres et du bec[5,6] et avec certains composés, comme le bidrin, toutes ces anomalies s'expriment[7–11]. Il a été montré que les anomalies du bec et des membres – prévenues par la nicotinamide[7–9,12] – n'étaient pas liées aux propriétés anticholinestérasiques de ces agents. Cependant malgré des observations préliminaires[11,13] aucune preuve formelle n'est venue à ce jour démontrer que les organophosphorés agissaient sur la morphogenèse vertébrale par l'intermédiaire d'un mécanisme spécifique. En relation avec ce problème, nous exposerons ici les résultats d'expériences ayant consisté à injecter, simultanément à deux insecticides organophosphorés (le parathion et le bidrin), de la pralidoxime, molécule connue et employée en clinique pour ses propriétés réactivatrices des cholinestérases phosphorylées[14,15]. Les effets de la pralidoxime seront comparés à ceux obtenus après l'emploi de la nicotinamide.

Matériel et techniques. Les œufs de caille (*Coturnix coturnix japonica*) utilisés sont issus de l'élevage du laboratoire. Les traitements tératogènes sont appliqués avant la mise en incubation des œufs. Le parathion purifié à 96,3% (Rhône-Poulenc) est dissous dans l'huile d'olive et injecté à raison de 75 µg par œuf. Le bidrin (dicrotophos; isomère *cis*-crotonamide), purifié à 85,8% (Schell) est dissous dans du soluté physiologique et chaque œuf reçoit 0,25 mg de ce composé. Les contre-traitements sont réalisés au 3e jour de l'incubation. La pralidoxime (sulfométhylate d'hydroxyiminométhyl-2