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Inhibition of Prostaglandin Synthesis in vivo by Nonsteroid Anti-Inflammatory Drugs: Evidence for the Importance of Pharmacokinetics

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Abstract

A variety of acidic and non-acidic compounds are potent inhibitors of prostaglandin (PG) synthesis in vitro. However, only a few, namely the acidic nonsteroid anti-inflammatory drugs (NSAID) are useful anti-inflammatory analgesics in the clinic. Since inhibition of PG-synthesis is believed to be the main target of NSAID in inflammation this superiority of acidic compounds remains unexplained. We have considered that one explanation could be that only acidic NSAID appear in high concentrations in inflamed tissue to inhibit PG-synthesis sufficiently.

To test this hypothesis the following experiments were carried out:

(A) PG-synthesis and its inhibition by acidic and non-acidic NSAID was measured in vivo at the site of inflammation. It was found that in therapeutic doses only acidic NSAID were capable to reduce PG-synthesis significantly.

(B) Measurement of drug concentration in inflamed tissue showed that only acidic NSAID were found in significantly higher concentrations in inflamed than in control tissue.

From these observations it is concluded that a specific pharmacokinetic behaviour of acidic NSAID leading to high concentrations in inflamed tissue is a decisive aspect of their anti-inflammatory action.

Introduction

Aspirin and aspirin-like nonsteroid anti-inflammatory drugs (NSAID) still belong to the most widely distributed and most frequently used drugs [1]. However, even today, in spite of tremendeous research efforts their mode(s) of action in inflammation remains abscure or at least controversial [2]. Reviewing the literature of the last decades it appears as if NSAID hide the essential aspect(s) of their anti-inflammatory action by being effective in almost every biological system in which they are tested. Thus the introduction of a new experimental system or the discovery of a new mediator of inflammation always gave rise to a new interpretation of their mode of action. For example, they have been shown to inhibit oxidative phosphorylation [3], histamine release [4], protein aggregation [5], lysosomal labilisation [6], leucocyte migration [7], erythrocyte lysis [8], lymphocyte stimulation [9], platelet aggregation [10], the potassium permeability of neuronal membranes [11] and the synthesis of prostaglandins (PG) [12]. All these observations gave rise to new speculations on the mode of action of NSAID. Some of these speculations never found wide acceptance and others were abandonned with time because new observations cast doubts as to the intrinsic importance of the former findings [1]. Even the PG-theory [12] which is currently very popular, leaves crucial questions unanswered.

Firstly, at present a broad variety of drugs ranging from acidic [13, 14] and non-acidic [14] anti-inflammatory drugs to psychotropic drugs [15] and local anaesthetics [16] have been found to inhibit PG-synthesis in vitro. Nevertheless, only a small group of acidic (pK_a around 4) drugs, which are bound to an unusually high degree to plasma protein under therapeutic conditions, have proven useful as anti-inflammatory agents in the clinic [1, 16].

Secondly, another question closely connected to the former concerns the common side effects of all acidic NSAID in use. All these drugs cause alterations in stomach and kidney which, depending on the dose given, range from irritation and functional impariment [12] to vast necrosis of cellular components in these organs [17]. It has been argued that both effects might

be due to inhibition of PG-synthesis in these organs because PG's appear to be physiological inhibitors of secretion of hydrochloric acid in the stomach [18] and might also regulate electrolyte elimination and regional blood flow in the kidney [19]. But why do other inhibitors of PG-synthesis not cause these effects?

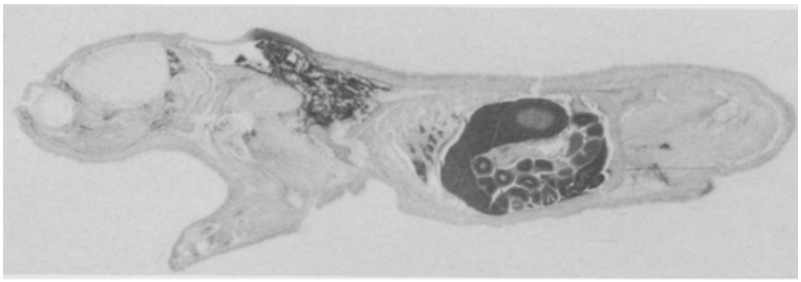
Thirdly, it should be mentioned that one outstanding effect of the pharmacology of acidic NSAID cannot be explained satisfactorily on the basis of the PG-theory [2]. In contrast to most other drugs the anti-inflammatory potency of NSAID appears to be directly correlated with the degree of albumin binding (interaction) in vivo, e.g., in acute joint synovitis [20]. Correspondingly, all useful NSAID are bound to plasma proteins to a high degree, usually around 90% after administration of therapeutic doses [13].

These unanswered questions prompted us to consider a further pharmacokinetic concept which together with the widely accepted pharmacodynamic theory(ies) could solve some of the remaining problems of the anti-inflammatory action(s) of NSAID.

The pharmacokinetic concept

We speculated that the two common physico-chemical characteristics of the useful NSAID, namely, their pK_a values around 4 [11, 21, 22] and their high degree of binding to plasma proteins in vivo [13], might cause high concentrations of these drugs in inflamed tissue but possibly also in certain cells of the stomach and kidney. High concentrations in these compartments could then cause impaired cellular function giving rise to, e.g., reduced pain perception in inflamed tissue and also to cell damage as found in the mucosa of the stomach and tubulus epithelia in the kidney [17].

To test this hypothesis we performed radio-autographic studies. When ^{14}C-labelled



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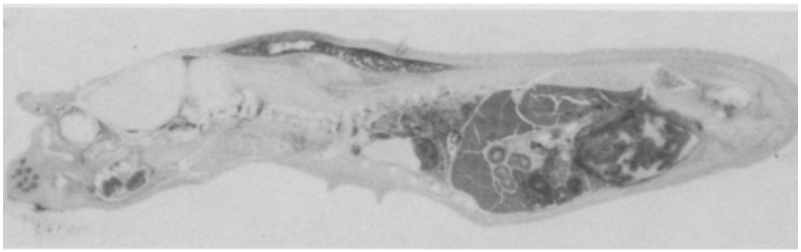

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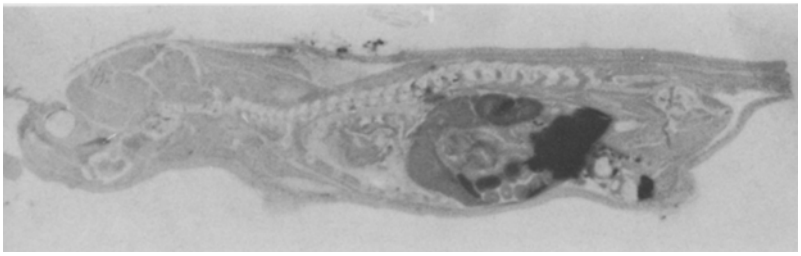

c

Radioautographs of phenylbutazone (a) indomethacin (b) and antipyrine (c) treated rats.
Young rats (30 g body weight) were given 100 Ci (10 mg/kg) ^{14}C-labelled phenylbutazone indomethacin or antipyrine by stomach tube. At the same time inflammation was elicited by the injection of 0.05 ml of a 2% (w/v) suspension of carrageenin into the left hind-paw of 0.2 ml suspension plus 0.2 ml air into the subcutaneous tissue of the neck. 5 hours later the animals were exsanguinated, deep frozen and cut into thin (100) slices which were mounted on X-ray film. After 8 days of exposure the pictures given were examined. The phenylbutazone or indomethacin treated animal (a and b) show high activity in the inflamed tissue of the neck and the left hind-paw whilst the antipyrine treated (c) does not display high activity in the inflamed tissue.

Table 1
Acidic and non-acidic pyrazolone and indole derivatives: Inhibition of PG-Synthesis and drug content in joint fluid and plasma 2 hours after eliciting an urate arthritis. The amount of drug calculated for 0.3 ml plasma is given because the joint washes contained approximately 0.3 ml joint fluid.

DRUG		ACIDITY	DOSE i.v. (mg/kg)	PG F_{2A}-CONTENT (% OF CONTROLS)	AMOUNT OF DRUG (ng) FOUND IN : PLASMA (0,3ml)	JOINT WASH CONTROL	JOINT WASH INFLAMED
PYRAZOLONE DERIVATIVES	ANTIPYRINE	ALKALINE pK_A 1,4	200	51±26,1	27,3±8,8	14,8±1,1	18,0±5,0
	PHENYLBUTAZONE	ACIDIC pK_A 4,4	20	39±13,6	2,6±0,9	0,7±0,2	3,0±1,1
INDOLE DERIVATIVES	INDOXOLE	ALKALINE pK_A 2,0	50	23±13,3	0,41±0,06	0,16±0,03	0,19±0,02
	INDOMETHACIN	ACIDIC pK_A 4,2	5	25±22,0	0,55±0,36	0,20±0,1	0,72±0,33

The drugs were dissolved in DMSO and infused slowly (10 minutes) i.v. at zero time. 1 hour later urate crystals (UC) were injected (4% w/v in saline) into the right intertarsal joint of the chicken (2 kg body weight) the left joint receiving 0.3 ml saline as a control. 3 hours later joint washes were performed, the PG F_{2a} content measured in the UC injected joints as described previously [33] and the drug dependent inhibition of PG-synthesis expressed in percent of DMSO treated controls. In other animals having received the same treatment the drug content in the inflamed and control joints was measured 3 hours after drug administration by fluorometric methods (antipyrine and indoxole) [34, 35] or using ^{14}C-labelled drugs. The values for 0.3 ml plasma are given because a joint wash contained about 0.3 to 0.4 ml fluid, i.e., not much more than was injected two hours before. Means and standard deviations of 5 or more experiments are given, ** $p < 0.01$.

phenylbutazone, indomethacin or antipyrine was administered to young rats and, at the same time, an inflammation elicited by the injection of carrageenin into the left hind-paw and the subcutaneous tissue of the neck, radio-autographs as given in the Figure were obtained. With the acidic NSAID they show high radioactivity in the inflamed tissue of the neck and the left hind-paw, but also in the stomach, small intestine and kidney. In contrast, experiments using ^{14}C-labelled antipyrine (see Figure) do not show accumulation of activity in the inflamed tissue indicating, as hypothesized before, that only acidic NSAID accumulate in inflamed tissue.

To get more quantitative information on this process we measured the concentration of different acidic NSAID and their non-acidic congeners in the synovial fluid obtained from inflamed and non-inflamed joints of chickens at different times after drug administration. The results for two indole derivatives and two pyrazolones are given in Table 1. Clearly, there is accumulation of the two acidic NSAID in inflamed joints. The concentration of these drugs in the fluid of these joints was already about three times higher than in the control joints at 3 hours, and exceeded the concentrations measured in the blood plasma at the same time. On the other hand the non-acidic congeners did not show such behaviour. The concentration of these drugs was equal or only slightly higher in the fluid of the inflamed as compared with the control joints and did not reach as high concentrations as in the plasma. Correspondingly, five to ten times higher doses of the non-acidic drugs were necessary to measurably inhibit PG-synthesis in vivo despite almost equal effectiveness of both derivatives in vitro [13, 14], indicating the importance of pharmacokinetics for the anti-inflammatory action of the acidic NSAID.

Possible explanations for the pharmacokinetics of NSAID

An explanation for this pharmacokinetic behaviour could be based on the two common

Table 2
Influence of variation of pH on the relative concentrations of sodium salicylate or phenylbutazone in neighbouring compartments.

DRUG	SITUATION	EXTRACELLULAR SPACE pH	EXTRACELLULAR SPACE CONCENTRATION	CELL MEMBRANE CONCENTRATION	INTRACELLULAR SPACE CONCENTRATION	INTRACELLULAR SPACE pH
SODIUM SALICYLATE	A	7.4	1.0	0.09	0.85	7.0
	B	6.8	1.0	0.12	1.2	7.2
PHENYL BUTAZONE	A	7.4	1.0	5.8	0.5	7.0
	B	6.8	1.0	15.8	2.0	7.2

It is assumed that the cell membrane behaves like octanol, the normally alkaline pH of the extracellular space (A) becomes acidic in inflamed tissue [27] (B) and the normally neutral intracellular pH (A) becomes slightly alkaline (B) in cells of inflamed tissue, e.g., in the non-lysosomal intracellular space of phagocytic granulocytes [36]. The values were defined by equilibrium distribution of phenylbutazone between citrate buffers (ionic strength 0.15 *M*) of different pH values and octanol. The phases were always saturated against each other.

physicochemical characteristics of acidic NSAID, namely their pK_a values around 4 and their high degree of binding to plasma proteins in vivo. The latter appears to be of great importance as indicated already by the observation that the clinical anti-inflammatory effect of NSAID in arthritis correlates directly with the degree of binding to plasma proteins in man [20]. How important the pK_a value is may be seen from Table 2. When one measures the degree of drug accumulation in the inflamed joint compared with blood plasma and control joints using acidic drugs of different pK_a values it appears as if a pK_a between 4 and 5 is optimal. Drugs being more acid or less acid do not accumulate to a comparable extent.

Using these observations, and taking into account the physiology of inflammation, gastric absorption and renal excretion of (acidic) compounds, the following explanatory hypothesis could be put forward:

Firstly, capillary damage with extravasation of plasma proteins in inflammation [23], intensive absorption of acidic compounds in the stomach [24] and active secretion followed by passive back diffusion of acidic compounds in the renal tubules [25] may cause high concentrations of these drugs in the extracellular space of these tissues.

Secondly, relative acidic pH values in the extracellular space, in comparison with alkaline intracellular pH values, are likely to cause a shift of acidic compounds (pK_a less than 7.0) into cell membranes and the intracellular space as exemplified in Table 3. In contrast weak acids (pK_a values of more than 7.0, e.g., phenobarbital) have already been observed to leave the intracellular space under acidic extracellular conditions [26]. This shift of acids (pK_a less than 7) into cell membranes and intracellular space is likely to be more pronounced with strong acids if the extracellular pH reaches very low values as e.g., in the stomach. This might explain the high incidence of side effects in the stomach seen with aspirin (pK_a 3.5) as compared with other NSAID. The same effect is likely to be more significant with weak acids (pK_a between 4 and 6) when the extracellular pH reaches only slightly acidic conditions as e.g., in inflammation (compare Table 3).

From these observations and speculations it is concluded that the situation found in

Table 3
Relative accumulation of weak acids in the fluid of arthritic joints.

DRUG	ACIDITY pKa	$\frac{C_{INFL.JOINT}}{C_{PLASMA}}$	$\frac{C_{INFL.JOINT}}{C_{CONTROL\ JOINT}}$
SODIUM SALICYLATE	3.0	0.91	1.5
PROBENECID	3.4	0.96	2.3
INDOMETHACIN	4.2	1.27	3.5
PHENYLBUTAZON	4.4	1.15	4.3
PHENOBARBITAL	7.2	0.49	1.27

The drugs were dissolved in saline or DMSO (when necessary) and infused slowly (10 minutes) i.v. at zero time. 1 hour later urate crystals (UC) were injected (4% w/v in saline) into the right intertarsal joint of the chicken (2 kg body weight) the left joint receiving 0.3 ml saline as a control. 3 hours later joint washes were performed as described previously [33]. The drug content in the joint washes was measured 3 hours after drug administration by photometric (sodium salicylate [37], probenecid [38]) methods or using ^{14}C-labelled compounds. The relative concentrations given are calculated from means of 5 experiments (compare Table 1).

inflamed tissue (acidic extracellular space [27], in stomach and probably also in kidney (alkaline interior of H^+ secreting cells [28, 29]) probably leads to higher concentrations of the acidic NSAID in these tissues at the site of possible pharmacodynamic actions, i.e., the cell membrane(s) or the intracellular space. The degree of concentration seen appears to be closely related to the local microenvironment, i.e., the local pH situation. Whether these explanations are sufficient to explain the observed accumulation and pharmacodynamic activities of acidic NSAID remains a matter for speculation at present [30].

Conclusion

It is still an unsettled question as to how NSAID exert their local anti-inflammatory action. However, it is quite clear that these drugs can interfere with a variety of cell functions, e.g., the generation and/or action of PG's and other mediators of inflammation. Our results together with previous observations [31, 32] clearly show that acidic NSAID reach higher concentrations in inflamed tissue than in most others throughout the body. Only in the absorptive and excretory organs, stomach, small intestine and kidney, can similar high concentrations be seen. This may cause the common side effects of all acidic NSAID in these organs and it could explain why all attempts to develop acidic NSAID without these side effects have been unsuccessful so far.

Acknowledgment

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References

[1] R.L. Miller and K.L. Melmon, *Inflammatory Disorders,* in: *Clinical Pharmacology* (MacMillan, New York 1972), p. 392.

[2] H.E. Paulus and M.W. Whitehouse, *Nonsteroid Anti-Inflammatory Agents,* Ann. Rev. Pharmac. *13,* 107 (1973).

[3] S.S. Adams and R. Cobb, *A Possible Basis for the Anti-Inflammatory Activity of Salicylates and other Non-Hormonal Anti-Rheumatic Drugs,* Nature *181,* 773 (1958).

[4] I.F. Skidmore and M.W. Whitehouse, *Biochemical Properties of Anti-Inflammatory Drugs,* VIII, Biochem. Pharmac. *15,* 1965 (1966).

[5] N.H. Grant, H.E. Alburn and C. Kryzanauskas, *Stabilization of Serum Albumin by Anti-Inflammatory Drugs,* Biochem. Pharmac. *19,* 715 (1970).

[6] L.J. Ignarro, *Dissimilar Effects of Anti-Inflammatory Drugs on Stability of Lysosomes from Peritoneal and Circulating Leukocytes and Liver,* Biochem. Pharmac. *20,* 2861 (1971).

[7] P. Phelps and D.J. McCarty, *Suppression of Crystal-Induced Synovitis in Canine Joints by Indomethacin.*

Demonstration of Inhibition of Leukocyte Mobility, Arthritis Rheum. *9,* 532 (1966).

[8] J.H. Brown, H.K. Mackey and D.A. Riggilo, *A Novel in vitro Assay for Anti-Inflammatory Agents Based on Stabilization of Erythrocytes,* Proc. Soc. exp. Biol. Med. *125,* 837 (1967).

[9] M.W. Whitehouse, *The Molecular Pharmacology of Anti-Inflammatory Drugs: Some Possible Mechanisms of Action at the Biochemical Level,* Biochem. Pharmac. Suppl., p. 293 (1968).

[10] M.B. Zucker and J. Peterson, *Inhibition of Adenosine Diphosphate-Induced Secondary Aggregation and other Platelet Functions by Acetylsalicylic Acid Ingestion,* Proc. Soc. exp. Biol. Med. *127,* 547 (1968).

[11] H. Levitan and J.L. Barker, *Salicylate: A Structure Activity Study of its Effects on Membrane Permeability,* Science *176,* 1423 (1972).

[12] S.H. Ferreira and J.R. Vane, *New Aspects of the Mode of Action of Nonsteroid Anti-Inflammatory Drugs,* Ann. Rev. Pharmac. *14,* 57 (1974).

[13] R.J. Flower, *Drugs which Inhibit Prostaglandin Biosynthesis,* Pharmac. Rev. *26,* 33 (1974).

[14] E.A. Ham, V.J. Cirillo, M. Zanetti, T.Y. Shen and F.A. Kuehl, *Studies on the Mode of Action of Non-Steroidal Anti-Inflammatory Agents,* in: *Prostaglandins in Cellular Biology,* (Plenum Press, New York, London 1972, p. 345.

[15] R.E. Lee, *The Influence of Psychotropic Drugs on Prostaglandin Biosynthesis,* Prostaglandins *5,* 63 (1974).

[16] H. Kunze, E. Bohn and W. Vogt, *Effect of Local Anaesthetics on Prostaglandin Biosynthesis in vitro,* Biochim. biophys. Acta *360,* 260 (1974).

[17] L. Arnold, C. Collins and G.A. Starmer, *Renal and Gastric Lesions after Phenylbutazone and Indomethacin in the Rat,* Pathology *6,* 303 (1974).

[18] A. Bennet, I.F. Stamford and W.A. Unger, *Advances in the Biosciences* (Pergamon Press-Vieweg, Braunschweig 1973), p. 265.

[19] J.B. Lee, *The Antihypertensive and Natriuretic Endocrine Function of the Kidney: Vascular and Metabolic Mechanisms of the Renal Prostaglandins,* in Prostaglandins in Cellular Biology (Plenum Press, New York 1972), p. 399.

[20] N.H. Grant, H.E. Alburn and C.A. Singer, *Correlation between in vitro and in vivo Models in Anti-Inflammatory Drug Studies,* Biochem. Pharmac. *20,* 2137 (1971).

[21] A. Goldstein, L. Aronow and S.M. Kalman, *Principles of Drug Action* (Harper and Row, New York 1969).

[22] H. Terada, S. Muraoka and T. Fujita, *Structure-Activity Relationships of Fenamic Acids,* J. med. Chem. *17,* 330 (1974).

[23] T.J. Williams and J. Morley, *Measurement of Rate of Extravasation of Plasma Protein in Inflammatory Responses in Guinea-Pig Skin Using a Continuous Recording Method,* Br. J. exp. Pathol. *55,* 1 (1974).

[24] C.A.M. Hogben, D.J. Tocco, B.B. Brodie and L.S. Schanker, *On the Mechanism of Intestinal Absorption of Drugs,* J. Pharmac. exp. Ther. *125,* 275 (1959).

[25] J.M. Weiner and G.H. Mudge, *Renal Tubular Mechanisms for Excretion of Organic Acids and Bases,* Am. J. Med. *36,* 743 (1964).

[26] W.J. Waddell and T.C. Butler, *The Distribution and Excretion of Phenobarbital,* J. clin. Invest. *36,* 1217 (1957).

[27] N.A. Cummings and G.L. Nordby, *Measurement of Synovial Fluid pH in Normal and Arthritic Knees,* Arthritis and Rheumatism *9,* 47 (1966).

[28] W.J. Waddell and R.G. Bates, *Intracellular pH,* Physiol. Rev. *49,* 285 (1969).

[29] K. Miyao, J. Physiol. Soc., Japan *29,* 18 (1967).

[30] K. Brune, *How Aspirin might work: A Pharmacokinetic Approach,* Agents and Actions *4,* 230 (1974).

[31] G. Wilhelmi and R. Pulver, *Untersuchungen zur Frage eines peripheren Angriffspunktes der Pyrazole bei der antiphlogistischen Wirkung,* Drug. Res. *5,* 221 (1955).

[32] A. Benakis, G. Tsoukas and B. Glasson, *Localization of Butylmalonic Acid-mono (1,2-diphenylhydrazide)-calcium^{14}C (Bumadizone-calcium), a New Anti-Inflammatory Drug, in Rat and Mice with Whole-Body Autoradiography,* Drug Res. *23,* 1231 (1973).

[33] M. Glatt, B. Peskar and K. Brune, *Leukocytes and Prostaglandins in Acute Inflammation,* Experientia *30,* 1257 (1974).

[34] D.G. Kaiser, B.J. Bowman and A.A. Forist, *Fluorometric Determination of Indoxole in Biological Specimens,* Analyt. Chem. *38,* 977 (1966).

[35] B.B. Brodie, J. Axelrod, R. Sobermann and B.B. Levy, *The Estimation of Antipyrine in Biological Materials,* J. biol. Chem. *179,* 25 (1949).

[36] K. Kakinuma, *Metabolic Control and Intracellular pH during Phagocytosis by Polymorphonuclear Leukocytes,* J. Biochem. *68,* 177 (1970).

[37] B.B. Brodie, S. Udenfriend and A.F. Coburn, *The Determination of Salicylic acid in Plasma,* J. Pharmac. exp. Ther. *80,* 114 (1944).

[38] E.K. Tillson, G.S. Schuchardt, J.K. Fishman and K.H. Beyer, *The Determination of Probenecid (Benemid) in Body Fluids,* J. Pharmac. exp. Ther. *III,* 385 (1954).

Discussion

Bonta (Netherlands)

I am interested in these urate-induced experiments. With urate crystals you will get a kinin release and also you will get a drop in the pH because there is a lot of lactic acid released by the crystals. When you give simultaneously colchicine, the colchicine will stop the mitotic phase and it also stops this drop in the pH, i.e. the lactic acid production. I am also wondering if you got accumulation of these compounds in colchicine treated animals? That is when you have no acid environment.

Brune (Switzerland)

Thank you Dr. Bonta. I am very sorry but radioautographs take a while, and I must admit that these experiments are made, but I cannot show pictures. I am sure you are right.