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Project for a study on regular intake of analgesics in women 30–49 years old[1,2]

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A prospective, longitudinal epidemiological study in various industries of Northwestern Switzerland

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Summary

The project and execution of an epidemiological long-term study from 1968 to 1972 on the regular intake of analgesics in 1250 working women and the age group of 30 to 49 years in 80 companies in Northwestern Switzerland are described in detail. The purpose of the study is to estimate the risk of urorenal disease. A preliminary study in 1965 showed that working women in middle age are inclined to the regular intake of analgesics. The general procedure with a screening phase, evaluation phase, control phase and completion after 5 years with questionnaires, blood and urine tests is described. In the appendix the analytic methods used are mentioned.

1. Purpose

The purpose of this study is to estimate the risk of developing urorenal illness in women 30–49 years of age who regularly use phenacetin containing analgesics or other types of analgesics as compared with that risk in women of the same age who use analgesics less regularly or not at all.

2. Background

The many autopsy and clinical studies indicating a possible relationship between regular intake of phenacetin containing analgesics and kidney disease have motivated a transversal epidemiological study sponsored in part by WHO, Geneva, Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung, Berne; F. Hoffmann-La Roche & Co. AG, Basle; Farbenfabriken Bayer AG, Leverkusen, W-Germany, and Burroughs Wellcome Co. Inc., Research Triangle Park, N. C. 27709, USA, and conducted in 1965 under my direction (Proceedings 3rd International Congress of Nephrology, Washington, D.C. 1966, Karger Basle-New York, Vol. II, p. 300, 1967, and Helv. Med. Acta *34*, 297 [1968]). This study in ten watchmaking factories in Northwestern Switzerland has indicated that women with evidence of N-Acetyl-p-aminophenol (= NAPAP = main metabolite of phenacetin) in their urine show higher prevalence of urinary tract abnormalities or disease than women showing no evidence of NAPAP in their urine. The objective parameters of urorenal system abnormality used in this study have been: Presence of significant bacteriuria, blood in urine, proteinuria, elevated serum creatinine or combinations of two or more of these. In addition the women with NAPAP in their urine have shown higher prevalence of kidney or urological disorders as judged by subjective evidence from the women's own statements. The study has also estimated that approximately 17 % of these women are regular users of pills containing phenacetin using the criterion of taking analgesics on two or more occasions per week by the person's own admission as subjective evidence of regular intake of phenacetin containing pills.
Because this pilot study of 1965 was of a point prevalence type, it could not answer the question of whether the risk of developing urorenal disease is greater in regular users of analgesics containing phenacetin than in a comparable group of women who are not abusers of such pills. This high prevalence of regular intake and the relationship between regular intake and urinary tract pathology shown in the data of this first study

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[2] *Consulting Panel:* Prof. Dr. P. Miescher (chairman), Geneva; Prof. E. Grandjean, ETH, Zürich; Prof. O. Gsell, Basle; Prof. H. Friebel, WHO, Geneva; Dr. E. H. Kass, Boston; Prof. P. Kielholz, Basle; Prof. H. Löffler, Basle; Prof. M. Schär, Zürich; Dr. L. F. Prescott, Edinburgh/Scotland; Prof. A. Studer, Basle, and PD J. Raaflaub, Basle.

[3] *Statistical work* prepared by P. S. Levy, Sc.D., Channing Laboratory, Harvard Medical School, Boston, Mass. 02118.

along with the autopsy and clinical evidence of this relationship from other investigations made it desirable to design a second, the present study, which attempts to answer the question of whether or not regular use of analgesics leads to urinary tract illness.
The question remains as to whether studies of early functional impairment in patients taking regularly analgesics can throw any light on the statement that continued intake of analgesics predisposes to infection by damaging the kidney. The difficulties inherent in such a work are obvious. First one has to determine accurately the incidence of pyelonephritis by age and sex in the population. Second one needs to show that the incidence of pyelonephritis or interstitial nephritis is higher in the group taking analgesics. Here large numbers are required to be quite certain of avoiding errors in such sampling, e. g. it may be that the group taking analgesics shows a higher incidence of renal tract infection before they ever started taking analgesics. It will be impossible to take a group of healthy young people who are not abusing analgesics and study their fate as they gradually begin to take analgesics in increasing doses.

3. General methodology

This is a selective (age, sex), longitudinal, prospective and cohort type of study to be executed in 3 phases, namely a *screening phase,* which will establish two groups to be followed for development of urinary tract illness, an *evaluation phase* and *follow-up phase.* In view of the difficulties to obtain positive information of the participants with regard to regular intake of analgesics, the study is based in the screening phase on objective criteria only.
Test group: consists of persons who show evidence of NAPAP in their urine during a prolonged screening.
Control group: consists of persons matched 1 : 1 with persons in the test group. Persons in the control group must show no evidence of NAPAP in their urine.
Both groups will be followed and examined yearly for evidence of urinary tract pathology or illness and according to the ruling of the Consulting Panel (20. 9. 68 Geneva) for additional metabolites of analgesics (Aminophenazon, Persedon) in urine. A final examination is planned 5 years from the beginning of the study. The incidence of urorenal disease will then be assessed for each group and the two compared. Details of this study will be outlined in the following sections.

4. Procedure

a) First screening with an initial questionnaire

(November 1967 to October 1968)
The population to be screened consisted of females born 1918–1937 from 88 single enterprises in Northwestern Switzerland (1970: 79). The industries represented were 5 chemistry (1970: 5), 10 department stores (1970: 8), 3 shoe (1970: 2), 7 alimentation + tobacco (1970: 5), 5 machines (1970: 4), 11 special machines and instruments (1970: 11), 5 bank and insurance (1970: 4), 12 textile (1970: 11), 26 watch (1970: 25), 4 paper (1970: 4). In 1970 17 women changed to another company which is already included in the study and 70 women left their former employer. This last number together with the 1969 number of 59 women form the groups 89–99. For convenience the enterprises were chosen from the area containing Basle–Le Locle–Zofingen–Baden (3,000 km²).
At the first meeting there was no interview with regard to regular analgesic intake nor to urorenal disease. The women were offered a health screening by letter for presence or absence of kidney disease and/or diabetes mellitus by the management of their employ-

Fig. 1 Procedure

Phases	Procedure	
Preparative phase (1967/68) Talk with management Personal director	Working women 20–65 years old (36,098) 30–49 years old (13,103) Voluntary participation (55 % = 7,311)	
Screening *1st Screening 1967/68* 3 urines examined for NAPAP short interview	1 sample or more positive for NAPAP A1 1,045 women	no sample positive for NAPAP B1
2nd Screening 1968 3 urines examined for NAPAP	1 sample or more positive for NAPAP 704 women	
1st Year 1968 Main examination and interview	*Test group: A2* NAPAP positive 623 women	*Control group: B2* no obj. and subj. evidence for regular intake of analgesics 621 women *Special group: B3* 14 women
2nd Year 1969 1st Control	Total = 1,231 women A2 = 611	B2 = 606 B3 = 14
3rd Year 1970 2nd Control	Total = 1,209 women A2 = 597	B2 = 600 B3 = 12
4th Year 1971 3rd Control		
5th Year 1972 4th and last Control and interview		

er. They were not informed on the second purpose of the study with regard to the evaluation of regular analgesic intake.
On the day of the first meeting one urine sample was collected from each woman and a short questionnaire completed. The questionnaire asked for a person's name, marital status, age, parity, place of work and nationality. This questionnaire was used for the matching procedure. All urines were analyzed with Labstix ®[1] for presence of sugar, protein, blood, ketone bodies and pH and the presence of NAPAP (in our laboratory) and Salicylates[2]. Two more urine samples were collected by the women on the following 2–3 days in the evening and sent to our laboratory in a prepared mail envelope and a plastic tube for determination of NAPAP and salicylates. Those persons with one or more specimens positive for NAPAP were placed in group A 1 (see fig.) while those having no positive specimen for NAPAP were placed in group B 1 for future reference.

b) 2nd screening

(January 1968 to November 1968)
All persons in group A 1 were summoned again at their place of work and asked to void urine once more immediately there, and twice at home in the following 2–3 days. The two samples collected at home were sent again by mail for determination of NAPAP and salicylates. Those persons having one or more positive specimens for NAPAP were placed in group A 2.

c) Main examination and interview
(April 1968 to December 1968)

All persons in group A 2 (test group) and B 1 (control group) were interviewed for information concerning their intake of analgesics.

[1] Kindly supplied by AMES Company, 6101 Seeheim (Bergstraße), W-Germany.
[2] Determined at Farbenfabriken Bayer AG, Leverkusen, W-Germany.

Those of group B 1 who claimed to take analgesics no more than once per week were placed in group B 2, a group of women without objective evidence of analgesic intake (NAPAP). Those two groups were asked questions (about 170) especially concerning their history of urorenal diseases and analgesic intake (see questionnaire: WHO interview 1967/68). A third group B 3 was formed with women from group B 1 admitting to take analgesics twice a week or more (this constitutes a special group without being matched with A 2). All three groups were asked to give blood specimens to be analyzed for creatinine. Urine was examined for bacteriuria (2×) as well as for specific gravity after thirsting overnight. A flat plate of abdomen was obtained whenever possible in the nearest hospital (done in 600 cases).

d) Matching for controls

Members from group B 2 were matched with members from group A 2 as follows:

1. Same age group:
1918–1922
1923–1927
1928–1932
1933–1937 (this criterion was always fulfilled).
2. Parity yes/no (criterion always fulfilled).
3. Nationality (Swiss – not Swiss: criterion was usually fulfilled).
4. Marital status (married, single, divorced, widowed, separated: this criterion was usually fulfilled).
5. Same kind of work (criterion was fulfilled whenever possible).

This matching was performed by the use of the questionnaire completed at the first screening. Each person obtained was interviewed according to the main examination.
In summary (see fig.) the screening procedure established two main groups: a *test group* (from A 2) as a study group of women

showing objective evidence of regular intake of analgesics containing NAPAP in urine; a *control group* B2 as a group of nonabusers (both by their own admission and by objective criteria) matched 1 : 1 with members of group A2 on variables believed to be of importance. In addition *group B3* is carried in the study. Its numbers show only subjective evidence for regular intake of analgesics; it is a small group (1969: 14 women).

5. Sample size

The sample size (see table) are those of type two error B = 15 %. They are maximal in that they are calculated for the comparison that requires the largest sample size that will be found at follow-up in group A2 and group B2.
As baseline data the 1027 women in the first study are used who had no metabolites of phenacetin or salicylates in their urine. This group had the following rates of renal and urinary tract abnormalities (rounded off to the nearest percentage).

Bacteriuria 7 % Hematuria 6 % Proteinuria 2 %
Creatinine 4 % One or more of the four 17 %

The question to be asked is the following: given the fact that from past experience, the rates of abnormality in women with no metabolites in their urine are as above; given a sample of 450 women in each group (5 years after initiation of the study) what is the minimal true incidence of these abnormalities that would have an 85 % probability of being detected by a statistical test performed at the 5 % level of significance? From calculations based on the method in section 8.13 of *Statistical Methods* (sixth edition by *Snedecor* and *Cochran,* Iowa State University Press) one would be 85 % certain of detecting a significant difference with 450 persons in each group if the true rates of abnormalities in the NAPAP positive group were as follows:

Bacteriuria 12 % Hematuria 11 % Proteinuria 5,4 %
Elev. Creatinine 8,3 % One more of the four 24 %

Thus it appears that a sample size at *450 women in each group at five years from the start* of the study is adequate to detect reasonable differences between the two groups. The tab. 1 is read as follows: If the attrition rate is 5 % per year, the original size of group A2 and B2 must be each 582 persons in order that there will be 450 persons available in each group 5 years later. It is anticipated that some of the individuals on the study group will be followed for less than 5 years because of the mobility of the population. Although every effort will be made to obtain as complete a sample as possible at the end of 5 years, there are statistical methods such as *Life Table Analysis (Reed L. J.* and *Merrell M.:* Amer. J. Hyg. *30,* 33 [1939]) which adjust for the fact that the length of follow-up is not the same for every patient *(cohort study).* This method will be used.

Table Sample needed to be screened in order to obtain 450 persons in group A2 and B2 at end of 5 years follow-up (type 2 error B = 15 %).

Annual Attrition	at 5 years	at 3 years	at 2 years	at 1 year	original group size	number needed for screening to obtain this size
1 %	450	459	464	468	473	2.955
5 %	450	498	525	552	582	3.635
10 %	450	556	617	685	762	4.763
15 %	450	623	733	862	1014	6.337
20 %	450	703	878	1099	1373	8.583

6. Evaluation phase

a) Follow-up examinations

A. Matching followed by main interview and examination at time 0.

B. Examinations and interviews in the 2nd (1969), 3rd (1970) and 4th year (1971).
C. Final control and repeat main interview in the 5th year (1972) including roentgenological control of kidney size.

b) Analysis

A. Transfer of data on IBM cards with the aid of the ZDV of Finanzdepartement Basel.
B. Computorial analysis and statistics by *Paul S. Levy* Sc. D. each year.
C. Termination of evaluation for a given matched pair will be made when the following changes are present in one of the two persons: 1. death, 2. loss to follow up or refusal (see reference to cohort study in section 5).

Appendix

The following *end points* will be used to evaluate regular analgesic intake and urorenal pathology:

A. Laboratory methods

a) Objective evidence for intake of analgesics

Determinations of metabolites were done in 6 urine samples for each person followed each year by 3 urine samples to the termination of the study.

1. Excretion of salicylates in urine

With the aid of a calorimetric, semiquantitative method put forward by the Firma Schweizerhalle (*Hanok A.:* Clin. Chemistry *8,* 400 [1962]) salicylates in urine can be determined quite specifically. After ingestion of salicylates a result of 20–49 mg % in a spontaneously voided urine can be considered as positive prove of intake. The determinations are performed in the laboratory of Dr. Lorke, Department of Toxicology, Farbenfabriken Bayer AG, Wuppertal-Elberfeld, W-Germany, under the care of Mrs. *Wirtz* M.D. All samples are deep frozen sent to Dr. *Lorke.* Results are stated 20–49 mg %, 50–99 mg % and greater than 100 mg %. All other samples are regarded as negative (0–19 mg %).

2. Excretion of N-acetyl-p-aminophenol in urine

can be detected after ingestion of phenacetin, N-acetyl-p-aminophenol and acetanilid by the method of *Welch* and *Conney* (Clin. Chem. *11,* 1064 [1965]). Normal values are 0.000 to 0.047 o.D. measured at 620 mμ. The method gives a normal result in casual urine specimens in about 50 % of cases after ingestion of the above compounds within 24 hours. It is always positive in 100 % of normal persons with 24 hour urine collection after ingestion of phenacetin containing pills.

The collection of 24 hour urines or 8 hour overnight urines is impossible in this type of study. An alternative is to collect the urine on 3 different days of one week to have a higher yielding of possible positive results without the difficulty of having the urine collected inadequately during a certain time period.

3. Chromatographic determination of aminophenazon derivates

The method of *Baeumler J.* and *Rippstein S.* (Pharm. acta Helv. *36,* 382 [1961]) is used to determine aminophenazon and one of the main metabolites, 4-amino-antipyrin, in each urine sample starting in 1969. Standards and control urines are done daily. Details of method: *Baumeler H. R.* and *Dubach U. C.* (Schweiz. Med. Wschr. *101,* 328–334 [1971]).

4. Chromatographic determination of pyrithyldion

Pyrithyldion = Persedon ® = 3,3-diethyl-2,4-dioxo-tetrahydropyridin is a component of

Saridon®. The method was developed in our laboratory in 1970 according to a proposition of Dr. *J. Raaflaub* (F. Hoffmann-La Roche & Co. AG, Basle); it is applied on the urine samples from 1969 onwards. Only urines positive for NAPAP are examined for pyrithyldion.

b) Objective evidence for urorenal disease

The effect of analgesics on the renal system and urinary tract were assessed with the following examinations:

1., 2. Urine for *blood, protein* with Labstix®. As for blood, all positive results were recorded as abnormal with the exception in women only during menstruation. As for protein, all results of 30 mg% or more are recorded as abnormal. *Glycosuria* is merely determined as a service to the persons examined. *pH* and *ketone bodies* were noted.

3. *Urine specific gravity:* This test can only be done when preparation of the patient is assured. An attempt was made to adhere to the following procedure: Women were not allowed to drink for at least 12 hours overnight. The person voided (clean void specimen) on arrival at the place of examination. The urine was saved for culture and for specific gravity (Refractometer AO). Values 1,010 or less were considered abnormal.

4. *Urine cultures* were obtained according to *Dubach* (Praxis *56,* 885 [1967]) as clean voided specimen and processed according to *Kass* (Ann. Int. Med. *56,* 46 [1962]) with the aid of the Institut für Mikrobiologie und Hygiene der Universität Basel (Direktor Prof. H. Löffler). All cultures were done in duplicate. Patients are called to be bacteriuric when 2 consecutive urine specimens taken within a 10–15 days period are positive ($\geq 10^5$ bacteria/ml) and show the same bacteria.

5. *Serum creatinine* was determined by an automated method (*Zender* and *Falbriard:* Clin. chim. Acta *12,* 183 [1965]) and assayed at the Med.-Chemisches Institut of the University of Berne (Dr. chem. *K. Lauber).* Blood was collected and stored at 4 °C uncentrifuged. The centrifugation was done at the laboratory of *Dr. Lauber* in Berne. Starting on 25th August 1969 all blood was centrifuged by the field team.
The following normal values were used: 0.01 to 1.50 mg%. Values of 1.51 mg% and more are considered as definitely abnormal in women. (*Dubach U. C.* and *Metz I.:* Klin. Wschr. *45,* 621 [1967].)

6. *Flat plate of the abdomen:* Flat plates at the distance of 1.20 m were taken. Pregnant women were excluded from X-rays. Size and volume of the kidneys are to be determined according to the method of *Ludin H.* (Acta radiologica *6,* 561 [1967]). Length and weight of all persons were measured (with clothes and shoes on).

B. *Interviews*

a) *First screening* with initial questionnaires: see card "WHO-Untersuchungen 1967/68".

b) *First examination* and interview: see "Ärztliche Untersuchungen unter Mithilfe der Weltgesundheitsorganisation WHO in Genf 1967/1968", which gives all details.

c) *Follow-up examinations* and interview each year for 3 years: see "Ärztliche Untersuchung unter Mithilfe der Weltgesundheitsorganisation WHO in Genf 1969, 1970, 1971", which gives all details.

d) *Final examination* and interview at end of study: Similar protocol with X-ray of kidney as under b.

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